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TITLE: Insulin Resistance, IGFs and Energy Balance on the Risk of Breast Cancer

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ABSTRACT

The purpose of this proposal is to research the association of insulin resistance and its joint effect with insulin like growth factors (IGFs) on breast cancer risk. Many epidemiological studies have investigated the association of body weight, fat distribution, and physical activity with the risk of breast cancer. Some studies have compared levels of C-peptide and IGFs between breast cancer cases and controls. None of these studies, however, has evaluated the potential joint effect of C-peptide, IGFs and energy balance on the etiology of breast cancer.

The specific aims of this proposal are 1) To determine blood levels of C-peptide and IGF1, IGF2 and IGFBP3 in a subset of subjects (400 case-control pairs) from the Shanghai Breast Cancer Study (SBCS) (R01CA64277) using pre-treatment blood samples and to evaluate the associations of blood C-peptide level and its joint effect with IGFs with the risk of breast cancer. 2) To analyze data collected from the SBCS to evaluate the association of energy balance with breast cancer risk. Evidence suggests that the combined effects of positive energy balance result in increased breast cancer risk and that C-peptide level is potentially related to the risk of breast cancer enhanced by IGF bioavailability.

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Body
Statement of Work

1c. Take 1 course in the Vanderbilt Department of Molecular Biology,: <u>Cancer Biology</u> (4 credits): Months 13-18.

Task 1. 1d. Take 1 course in the Vanderbilt Department of Molecular Physiology and Biophysics, (Fall Semester, 2003), <u>Molecular Endocrinology</u> (2 credits): Months 21-24.

Task 2. Undergo extensive research training in the aspect of the association of C-peptide, IGFs, and energy balance with breast cancer risk: Months 1-36.

2a. Analyze data from The Shanghai Breast Cancer Study (1500 cases and 1500 controls) to evaluate the association between energy balance and breast cancer risk and prepare a manuscript to report the findings: Months 1-8.

- Appendix 2: Malin et al, Abstract: Body weight and body fat distribution in relation to lifestyle factors among Chinese women in Shanghai, NAASO, 2001.
- Appendix 3: Malin et al, Intake of fruits, vegetables and selected micronutrients in relation to the risk of breast cancer. *Int. J. Cancer*, **105**, 413-418 (2003).

2b. Design a case-control study within the Shanghai Breast Cancer Study (400 cases and 400 controls) to evaluate the association of C-peptide, and IGFs with breast cancer risk and prepare blood samples for relevant assays: Months 1-6.

2c. Analyze and publish the relationship between C-peptide and breast cancer risk: Months 12-18.

2d. Analyze and publish the joint effect of C-peptide with IGFs on breast cancer risk: Months 15-20.

- Appendix 4: Malin et al, Abstract and corresponding poster presentation " Insulin Resistance, Insulin-Like Growth Factors and Breast Cancer Risk" for DOD Era of Hope Conference, Orlando, Florida 2002.
- Appendix 5: Malin et al, Evaluation of the synergistic effect of insulin resistance and insulin-like growth factors on the risk of breast carcinoma. *Cancer*, **100**(4): 694-700 (2004).

2e. Analyze and publish the relationship of diet, physical activity, body mass index (BMI), and waist-to-hip ratio (WHR) with C-peptide and IGFs: Months 20-36.

- Appendix 6: Abstract and corresponding poster presentation entitled "Energy Balance; C-peptide, Insulin-Like Growth Factors and Breast Cancer Risk" at NAASO/ADA, 2003.
- Appendix 7: Abstract and corresponding poster presentation entitled "Combined Association of Energy Balance, Lifestyle Factors and Breast Cancer Risk" for poster presentation at the 95th annual AACR meeting, 2004.
- Appendix 8: Malin et al, (2005) Energy Balance and Breast Cancer Risk. <u>Cancer Epidemiol Biomarkers Prev</u>;14(6) 1496-1501.
- Appendix 9: Sanderson et al, (2004). Insulin-like Growth Factor-I, Soyfood Intake and Breast Cancer Risk. <u>Nutrition & Cancer,</u> 50(1) 8-15.
- Appendix 10: Abstract and corresponding poster presentation entitled "Correlation of insulin-like growth factors and c-peptide with energy balance on breast cancer" for poster presentation at NAASO annual meeting, Vancouver, BC., 2005.

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- Appendix 11: **Malin, A.S.**, Dai, Q., Matthews, C.,Yu, H., Shu, X., Jin, F. Gao, YT, Zheng, W., Correlation between energy balance, IGF-I, IGFBP-3, C-peptide and endogenous sex hormones on breast cancer risk(unpublished data).

Task 3. Prepare grant proposal for continuation

    3a. Develop and submit a grant proposal to expand the sample size of the study to evaluate C-peptide, IGF, estrogen, and phytoestrogens in relation to breast cancer risk: Months 24-30.

- Appendix 12 : Specific aims of funded U54 proposal "Obesity, Insulin-Resistance, IGFs and Breast Cancer Risk in African-Americans" (U54CA091408-05)

Key Research Accomplishments

- **September 2001**: Completed <u>Biostatistics 1</u> course in the Department of Preventive Medicine.
- **October 2001**: Presented poster entitled "Body weight and body fat distribution in relation to lifestyle factors among Chinese women in Shanghai" at the North American Association for the Study of Obesity, Québec City, Canada.
- **January 2002**: Began designing case-control sub-study on IGF, C-peptide and breast cancer risk from the Shanghai Breast Cancer Study (parent study).
- **February 2002**: Completed <u>Biostatistics 2</u> course in the Department of Preventive Medicine.
- **March 2002**: Completed <u>Epidemiology 2</u> course in the Department of Preventive Medicine.
- **March 2002**: Coordinated delivery of 724 blood samples from the Vanderbilt Ingram Cancer Center laboratory to Dr. Herbert Yu (consultant) at Yale University Department of Epidemiology and Public Health, for relevant assay analysis.
- **April 2002**: Prepared manuscript entitled "Intake of fruits, vegetables, and selected micronutrients in relation to the risk of breast cancer "utilizing data from the Shanghai Breast Cancer Study for submission to the *International Journal of Cancer*.
- **April 2002**: Through collaboration with Dr. Herbert Yu in the Yale University Department of Epidemiology and Public Health, completed lab analysis on blood samples (528 C-peptide case controls pairs and 196 IGF1, IGF2, and IGFBP3 pairs) for relevant assays within the Shanghai Breast Cancer Study case-control study to evaluate the association of C-peptide, and IGFs with breast cancer risk.
- **April 2002**: Started analysis of joint effect of C-Peptide, IGFs and energy balance on breast cancer risk.
- **May 2002**: Completed <u>Grant Writing</u> course in the Department of Preventive Medicine..
- **May 2002**: Received biomarker assay dataset on IGF1, IGF2, IGFBP3, and C-peptide from collaborator, Dr. Herbert Yu, Yale University School of Medicine.
- **June 2002**: Submitted food group paper to *International Journal of Cancer*.
- **June 2002- August 2002**: Began cleaning of IGF dataset, formulation of tables and preliminary analysis of data.
- **September 2002**: Revised and resubmitted food group paper to *International Journal of Cancer*.
- **September 2002**: Presented the poster entitled "Insulin Resistance, Insulin-Like Growth Factors and Breast Cancer Risk" for the DOD Era of Hope conference in Orlando

Florida. **October 2002:** Interviewed for faculty position at the Department of Surgery, Meharry Medical College, candidate for Assistant Professor, Department of Surgery.

- **November 2002-December 2002:** Prepared first draft of IGF/ C-peptide joint association manuscript.
- **January 2003**: Food group paper accepted to *International Journal of Cancer.*
- **February 2003**: Finalized analysis of the joint effect of C-peptide with IGFs on breast cancer risk.
- **March 2003**: Prepared joint association manuscript for submission to the *British Journal of Cancer.*
- **April 2003**: Began analysis of the relationship of diet, physical activity, body mass index (BMI), and waist-to-hip ratio (WHR) with C-peptide and IGFs for abstract submission to the North American Association for the Study of Obesity/American Diabetes Association.
- **May 2003**: Submitted joint effect manuscript to the *British Journal of Cancer.*
- **May 2003:** Submitted abstract on the relationship of diet, physical activity, body mass index (BMI) and waist-to-hip ratio (WHR) with IGFs and C-peptide to the North American Association for the Study of Obesity/American Diabetes Association 2003 Annual Meeting.
- **May 2003:** Accepted job offer as Assistant Professor of Surgery, Department of Surgery, Meharry Medical College, Nashville, TN 37208.
- **August 2003:** Submitted joint effect manuscript to *Cancer.*
- **August 2003:** Began Assistant Professor position at Meharry Medical College.
- **September 2003:** Submitted a grant proposal to the Vanderbilt Ingram Cancer Center/Meharry Medical College Cancer Partnership Grant mechanism for a pilot project on minority women, mammography screening behaviors and anthropometric factors on breast cancer risk.
- **October 2003:** Presented the poster entitled "Energy Balance; C-peptide, Insulin-Like Growth Factors and Breast Cancer Risk" for the North American Association for the study of Obesity and American Diabetes Association (NAASO/ADA), 2003. **November 2003:** Submitted abstract entitled" Combined Association of Energy Balance, Lifestyle Factors and Breast Cancer Risk" to the American Association of Cancer Research.
- **December 2003**: Finalized analysis on combined association of energy balance on breast cancer risk.
- **January 2004**: Submitted manuscript as co-author with Maureen Sanderson entitled "Insulin-like Growth Factor-I, Soyfood Intake and Breast Cancer Risk" to *Cancer Causes and Control*, January 2004.
- **February 2004**: Received AACR-Minority-Serving Institution (MSI) Faculty Scholar Travel Award in Cancer Research from the American Association of Cancer Research for the 95th annual conference in Orlando, Florida to present poster entitled "Combined Association of Energy Balance, Lifestyle Factors and Breast Cancer Risk".

- **March 2004**: Received invitation from Cancer Epidemiology Biomarkers and Prevention to submit manuscript from corresponding poster to their journal
- **April 2004:** Prepared first draft of manuscript entitled "Energy Balance and Breast Cancer Risk".

- **April 2004:** Completed a chapter in a basic nursing text Epidemiology. In C.H. Yarboro, M.H. Frogge, M. Goodman, S.L. Groenwald (Eds.): <u>Cancer Nursing, Principles and Practice.</u> Jones and Bartlett Publications, Sudsbury, MA. 2005.
- June 2004:Soy food paper accepted to *Nutrition & Cancer*
- **July 2004:** Began formulation of tables for energy balance paper
- **September 2004:** Revised final analysis on combined association of energy balance on breast cancer risk.
- **November 2004:** Submitted manuscript entitled "Energy Balance and Breast Cancer Risk" to *Cancer Epidemiology Biomarkers & Prevention.*
- **December 2004:** Began data analysis for paper entitled "Correlation between energy balance, IGF-I, IGFBP-3, C-peptide and endogenous sex hormones on breast cancer risk"
- **January 2005:** Co-wrote a pilot project for the U54 Meharry Vanderbilt Cancer Partnership renewal entitled "Obesity, Insulin-Resistance, IGFs and Breast Cancer Risk in African-Americans"
- **March 2005:** Submitted abstract to North American Association for the Study of Obesity (NAASO) entitled "Correlation between energy balance, IGF-I, IGFBP-3, C-peptide and endogenous sex hormones on breast cancer risk"
- **May 2005:**Met with Herbert Yu, PhD, collaborator on postdoctoral award at Vanderbilt regarding IGFI-R pathway perturbation and type two diabetes risk for Komen Foundation talk in June 2005.
- **June 2005:** Presented <u>The Insulin Connection as a</u> panel speaker at the 8th Annual Mission Conference of the Susan B. Komen Foundation Plenary Session on Breast Cancer Prevention and Risk Reduction, Washington, DC, June 2005.
- **June 2005:** Energy balance paper published in *Cancer Epidemiology Biomarkers and Prevention*
- **June 2005:** Hired Jared Elzey, BA(15% effort) as a research assistant for the DOD postdoctoral research award to assist with manuscript and poster presentation preparation.
- **July 2005:** Presented progress of DOD grant to Vanderbilt Ingram Cancer Center Bi-weekly Epidemiology group "Correlation of insulin-like growth factors and C-peptide with energy balance on breast cancer risk"
- **August 2005:** Revised analysis to include endogenous estrogen data from Sonia Boyapati,PhD for the correlation manuscript.
- **August 2005:** Sent correlation paper abstract to Journal of Clinical Endocrinology and Metabolism, paper concept was rejected.

Reportable Outcomes

- The additional biostatistics and epidemiology training provided through the Vanderbilt Preventive Medicine department, Masters in Public Health program has enabled me to independently perform statistical analysis on SAS and Stata, coordinate and implement a five hospital case-control study in Nashville, Tennessee entitled the "Nashville Breast Health Study".
- I have presented an abstract at the North American Association for the Study of Obesity (NAASO) that examined the association between body fat distribution and lifestyle factors of women residing in Shanghai, China.

- I have designed and implemented a sub-study within the Shanghai Breast Cancer case-control study to evaluate the joint effects of C-peptide, and IGF1, IGF2 and IGFBP3 on breast cancer risk. In addition, the sub-study will function to enhance the statistical power of the parent study. The Shanghai Breast Cancer study has completed assays of C-peptide for 143 case-control pairs and IGF1, IGF2, and IGFBP3 for 300 case-control pairs. To enhance the statistical power of this study for evaluating joint effects of these biomarkers, this proposal has facilitated additional C-peptide assays for 514 (257 pairs) samples and 300 assays for an additional 200 samples (100 pairs) to bring the total sample size to 400 case-control pairs.
- I have presented a poster at the DOD Era of Hope meeting that examined the joint association between IGF1, 2 and binding protein 3 with C-peptide on breast cancer risk.
- I have a published manuscript in the *International Journal of Cancer* on the association of vegetable, fruit and vitamin intake with breast cancer risk using data from the Shanghai Breast Cancer case-control study.
- I have published a manuscript in *Cancer*, on the joint association of IGF-I, IGFBP-3 and C-peptide on breast cancer risk.
- I have presented a poster at the North American Association for the Study of Obesity/American Diabetes Association on the joint association of biomarkers IGF1, IGF2, IGFBP-3, C-peptide and the energy balance equation on breast cancer risk.
- I have co-authored a paper on soyfood intake IGF1 and breast cancer risk that has been published in Nutrition & *Cancer*.
- I have been appointed as an Assistant Professor of Surgery at Meharry Medical College as of August 1, 2003.
- I have been asked to write a basic epidemiology chapter for a nursing textbook distributed to master's level nurses worldwide.
- I have received funding for a pilot project as a Principal Investigator to conduct a case-control study on minority women and resolution of abnormal mammography findings through diagnostic procedures. The grant is entitled "Factors impacting delay in diagnostic mammography resolution in African-American Women in a public hospital setting (U54 CA91408-04).
- I have received a faculty scholar travel award from the American Association for Cancer Research to present my poster on the combined association of positive energy balance and breast cancer risk. Selection for this award was based on my research I have conducted with support from the DOD postdoctoral training award.
- I am on a national speaker's list for the Susan G. Komen Breast Cancer Foundation as a junior investigator in the field of insulin and breast cancer risk.
- I have been selected to be an ad-hoc reviewer for the journal *Nutrition and Cancer*.
- I am serving a co-investigator on the NCI funded project (U54CA091408-05) Obesity, Insulin-Resistance, IGFs and Breast Cancer Risk in African-Americans

Conclusions

- Results from the *International Journal of Cancer* manuscript revealed that there was no association between breast cancer risk and total vegetable intake. However, the risk of breast cancer declined with increasing intake of dark yellow-orange vegetables (trend test, p=0.02),Chinese white turnips (trend test, p≤0.001), and certain dark green vegetables (trend test, p≤ 0.001) with adjusted ORs in the highest quintile being 0.79

(95% CI =0.60-0.98) ,0.67(95% CI =0.53-0.85) and 0.65(95% CI=0.51-0.83) respectively. Intake of fruits, except watermelons and apples, was inversely associated with breast cancer risk (p value for trend test, ≤0.05). This study suggests that high intake of certain vegetables and fruits may be associated with a reduced risk of breast cancer.

- Results from my poster presentation on the joint association of blood C-peptide and IGFs 1,2 and BP3 and breast cancer risk in women in Shanghai, China reveal that the risks of breast cancer were increased with higher levels of C-peptide and IGFs in the blood, with significant dose-response relationships. There was a statistically significant, two to threefold increased risk of breast cancer for women in the highest quartile of C-peptide, IGF-I, or IGFBP-3 compared to women in the lowest quartiles.

- Results from the *Cancer* manuscript illustrate that women with high levels of both C-peptide and IGFs have a substantially higher risk of breast cancer than those with only a high level of C-peptide and IGF1 or IGFBP-3. The adjusted ORs were 3.79 (95% CI=2.03-7.08) for a higher level of both C-peptide and IGF1 and 4.03 (95% CI= 2.06-7.86) for a higher level of both C-peptide and IGFBP-3. These results confirm previous findings where a positive association of breast cancer risk with C-peptide and IGFs intimates that the above molecules synergistically increase the risk of breast cancer.

- Results from my poster presentation on energy balance, C-peptide and IGFs on breast cancer risk revealed that pre-menopausal women that did not engage in occupational activity and had higher BMIs had a two fold risk of breast cancer and postmenopausal women who did not engage in physical activity for the past ten years and had higher BMIs were at a five fold risk of breast cancer. C-peptide levels seemed to more closely track breast cancer associations across strata of energy balance than the IGF parameters. Positive energy balance may play a role in this association.

- The paper co-authored with Dr. Maureen Sanderson was a sub-study of the Shanghai Breast Cancer Study (PI: Dr. Wei Zheng). The analysis for this paper was restricted to the 397 cases and 397 matched controls for whom information on IGF-I levels was available as provided by postdoctoral training award DAMD17-01-0437 (PI: Alecia Malin). The results revealed for premenopausal breast cancer, a nearly significant negative interaction between soy protein intake and IGF-I levels (p=0.08), and a nearly significant positive interaction between soy protein intake and IGFBP-3 levels (p=0.06). No interaction was evident between soy protein intake and IGF-I or IGFBP-3 levels among postmenopausal women. The results suggest that soy protein intake may modify the effect of IGF-I and IGFBP-3 levels on breast cancer risk. Further studies are needed to confirm these findings and to understand the biological mechanisms of these interactions.

- Results from my poster presentation for AACR and manuscript for *Cancer Epidemiology Biomarkers and Prevention* on the combined association of energy balance, lifestyle factors and breast cancer risk found that a synergistic effect on breast cancer risk was suggested among pre-menopausal women in the highest quartile of body mass index (BMI) or waist-to-hip ratio (WHR) that were not occupationally active. Odds ratios were 2.24(95% CI=1.20-1.42) for women with both high BMI and no occupational activity,

3.24(95%CI=1.61-6.50) for women with high WHR that did not engage in occupational activity. Post-menopausal women who reported no physical activity, consumed high levels of calories and had large BMIs were at higher risk than those who were active, consumed low levels of energy and had low BMIs. The adjusted ORs were 3.51(95% CI=1.02-12.06) for post-menopausal women with a high BMI, no physical activity and low energy intake and 3.90(95% CI=1.13-13.47) for post-menopausal women with a high BMI, no physical activity and high energy intake. In conclusion, these results suggest that lifestyle factors, including higher central body fat distribution and overall adiposity, lack of physical activity and high caloric intake jointly convey an increased risk of breast cancer together than separately.

- Results from my poster presentation for NAASO and manuscript in preparation for *Cancer* on the correlation between energy balance, IGF-I, IGFBP-3, C-peptide and selected endogenous estrogens on breast cancer risk found that Body mass index and waist:hip ratio was significantly positively correlated with IGFBP-3 and C-peptide. Adult exercise/sport activity and occupational activity were significantly negatively correlated with IGF-I. Adolescent exercise/sport activity and energy intake were not associated with levels of insulin-like growth factors, binding protein 3 or C-peptide. There was a significant trend of increasing IGF-I levels with increasing quartiles of BMI. IGF-II levels increased with increasing quartiles of BMI. Both IGF-II and C-peptide increased with increasing quartiles of waist: hip ratio (p trend <0.01). Additional multivariate analyses were performed to evaluate the joint association of energy balance measures to the insulin-growth factor, C-peptide and endogenous sex hormone biomarkers. None of these results changed substantially after adjustment for each of the above biomarkers singly and in combination.

Conclusion: "So What" Category

- This second year of postdoctoral funding has enabled me to complete tasks 1.1b.and tasks 2.2a-2.2e. I am continuing to examine the role of biomarkers in breast cancer epidemiology and have found that IGF1 and IGFBP-3 separately with higher levels of blood C-peptide result in a three-fold risk of breast cancer. As a combined measure high levels of IGF1, IGFBP-3 and C-peptide have over a five-fold risk of breast cancer in post-menopausal women. This is the only epidemiology study to date that has examined the above joint association of biomarkers on breast cancer risk in such a large Asian population.

- This postdoctoral training award has enabled me to develop my career in molecular and lifestyle epidemiology. My doctoral training was in health promotion and education. My postdoctoral work from 2000-2003 at Vanderbilt Medical Center consisted of rigorous coursework in epidemiology and biostatistics. I was able to apply the didactic principles of my coursework in the design and implementation of a case-control study on the synergistic effect of blood C-peptide, Insulin-Like Growth Factors and anthropometric factors on breast cancer risk. I was given the opportunity to write manuscripts on my findings from this case-control study and present the results at scientific meetings. This

- postdoctoral work from 2000-2003 at Vanderbilt Medical Center consisted of rigorous coursework in epidemiology and biostatistics. I was able to apply the didactic principles of my coursework in the design and implementation of a case-control study on the synergistic effect of blood C-peptide, Insulin-Like Growth Factors and anthropometric factors on breast cancer risk. I was given the opportunity to write manuscripts on my findings from this case-control study and present the results at scientific meetings. This award has elevated my standing from a post-doctoral fellow to an Assistant Professor of Surgery, where I am collaborating with other faculty members at neighboring institutions on their respective research interests and the data on the blood biomarkers I collected from the post-doctoral training grant. I am now applying for extramural funding from federal and institutional grants as a Principal Investigator and examining breast cancer risk, anthropometric markers and biomarkers on minority populations in the public hospital setting. I am being asked to write chapters in textbooks from other disciplines on the basics of epidemiology, a skill I would have never been able to achieve with my doctoral degree alone. This postdoctoral training grant from the Department of Defense allowed me to train in a competitive high caliber environment under Dr. Wei Zheng, a mentor to several other DOD award recipients (Dr. Maureen Sanderson and Dr. Femi Adegoke) that continued in academia to become Associate and Assistant Professors, respectively, with independent research projects and extramural funding from federal grants.

References

1. **Malin, A.S.**, Matthews, C., Cai, H., Yu, H., Shu, X., Jin, F. Gao, YT, Zheng, W., Energy Balance: C-peptide, Insulin-Like Growth Factors and Breast Cancer Risk. (2003). <u>Obesity Research, 11: A130-A130 Suppl.S.</u>

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3. **Malin, A.S.**, Dai, Q., Yu, H., Shu, X., Jin, F., Gao,YT., Zheng, W. Evaluation of synergistic effect of insulin resistance and insulin-like growth factors on the risk of breast cancer.(2004) <u>Cancer,</u> 100(4) 694-700.

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5. **Malin, A.** (2005). Epidemiology. In C.H. Yarboro, M.H. Frogge, M. Goodman, L.. Groenwald (Eds.): <u>Cancer Nursing, Principles and Practice.</u> Jones and Bartlett Publications, Sudsbury, MA. 2005.

6. Sanderson, M., Shu, X., Yu, H., Dai, Q., **Malin, A.S.,** Gao,YT., Zheng, W.(2004). Insulin-like Growth Factor-I, Soyfood Intake and Breast Cancer Risk. <u>Nutrition &</u> <u>Cancer,</u> 50(1) 8-15.

7. **Malin, A.S.**, Matthews, C., Shu, X., Cai, H., Dai, Q, Jin, F. Gao, YT, Zheng, W. (2005) Energy Balance and Breast Cancer Risk. <u>Cancer Epidemiol Biomarkers Prev</u>;14(6) 1496-1501.

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9. **Malin, A.S.**, Dai, Q., Matthews, C.,Yu, H., Shu, X., Jin, F. Gao, YT, Zheng, W., Correlation between energy balance, IGF-I, IGFBP-3, C-peptide and breast cancer risk (In progress).

List of grant personnel receiving monetary compensation from award number
DAMD17-01-1-0437

1. Dr. Alecia Malin
2. Dr. Herbert Yu
3. Jared Elzey, BA

CANCER NURSING

PRINCIPLES AND PRACTICE

SIXTH EDITION

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Contents

Epidemiology

Alecia Malin, DrPH, CHES

Introduction

Cancer epidemiology examines the frequency of cancer in populations, the role of certain risk factors that contribute to cancer rates, and the interrelationships or associations that exist between the host, the environment, and other conditions that may contribute to the development or inhibition of cancer.[1] The basic premise of epidemiology is that disease does not occur randomly, but rather in describable patterns that reflect the underlying etiology, or causes of cancer. Because disease does not occur randomly, individuals who have cancer must have been exposed to some factor, either voluntarily (through diet, medication, or smoking), or involuntarily (through factors such as cosmic radiation, air pollution, occupational hazards, or genetic constitution that contributed to the causation of disease).[2] The application of epidemiology to cancer research allows investigators to identify possible causes of disease by elucidating how those exposed and not exposed to risk factors toward cancer differ.

The first section of this chapter reviews basic epidemiological concepts. These concepts will help the reader better understand epidemiologic research, identify groups at higher risk for cancer development, and learn how to conduct research in the field of cancer epidemiology. After reading this chapter, the reader should understand the major issues involved in cancer research design, assessment, and estimation of cancer risks. A brief glossary of fundamental terms used in the field of epidemiology is given in Table 3-1. Table 3-2 includes rates and ratios frequently calculated in epidemiologic research.

Subsequent sections discuss causes of cancer, risk factors that influence cancer susceptibility, and the application of epidemiologic principles in nursing practice.

Basic Considerations in Epidemiological Research

Six primary components are considered when evaluating an epidemiological research project:

1. Definitions of the disease and exposures related to the research hypothesis
2. Study design
3. Eligibility and exclusionary criteria used to select study participants
4. Definition of the source and study populations to be used in the study
5. Statistical plan measuring the association between the exposures and the disease

6. Identification of potential sources of bias and confounding variables[3]

Study Designs

Several standard study designs are used in epidemiologic research. Although this section discusses the general features of these designs, the primary emphasis is on the three designs most commonly used in epidemiologic cancer research: the case-control, cohort, and clinical trial study designs. Other major study designs include experimental, ecological, and cross-sectional.[4]

In selecting the appropriate study design, several factors must be considered:

- The frequency of the disease or the exposure in the general population and the defined population to be studied

- The length of the latency period

- The anticipated size of the study sample

- The time allowed for subject recruitment

- The diagnostic characteristics of the disease and the measurability of the exposure[3]

Case-control studies

The case-control study design should be considered if at least one of the following criteria is met:

- The disease is rare in the general or source population (many forms of cancer meet this criterion).

- The investigation is preliminary.

- Time and funding limitations prohibit the use of larger, more expensive study designs.

The hallmark of the case-control study (as illustrated in Figure 3-1) is that it begins with people with the disease (cases) and compares them to people without the disease (controls).[5] Subjects in case-control studies are recruited on the basis of their disease status. Cases of the disease in question can be either preexisting or newly developed. Generally, a strict definition of the disease is used to identify eligible subjects. For example, pathology slides, cytology results, or medical records can be examined to identify the stage or histology of a cancer. The control subjects, or noncases, are defined as participants who do not have the disease at present but who, if the disease did develop, would have the same opportunity to be diagnosed as the case subjects.

The assumption that cases and controls originate from the same hypothetical source cohort is a critical issue affecting the validity of case-control data. Both cases and controls must originate from populations

Table 3-1   Glossary of Epidemiological Terms

Association	Statistical association refers to the strength of the relationship between two variables. In epidemiological terms, association indicates the degree to which the rate of disease in persons with specific exposure is either higher or lower than the rate of disease in persons without the exposure. The strength of this dependence is greater than what would be expected by chance.
Bias	Selection bias results from a systematic difference in the manner in which the case and comparison groups are selected for participation in the study. This bias may produce spurious associations due to the differential inclusion or exclusion of subjects from the disease or exposure groups.
Case-control study	A study where individuals are selected according to the disease status of interest — those who have the disease (cases) and those individuals who do not have the disease (controls). The cases and controls are examined to ascertain which proportions were exposed to the disease risk factors and which were not.
Cohort study	A study where individuals are classified according to their exposure and are observed to ascertain the frequency of disease occurrence or death among those in various exposure-defined categories.
Confounding	The systematic overestimation or underestimation of the effect of an exposure because the influence of a disease risk factor has not been taken into account. A confounding variable is a risk factor for the disease being studied that is associated with the exposure being studied and is not an intermediate step between the exposure and the disease.
Epidemiologic triangle	The traditional model of infectious disease causation. It consists of three components: an external agent, a susceptible host, and a environment that brings the host and the agent together. Also known as the epidemiologic triad.
Epidemiology	Nonexperimental investigation of disease causation through observational study of human population groups. Descriptive epidemiology is the study of the frequency of occurrence of (incidence) or death from (mortality) in a disease population stratified by time, place, and/or group characteristics. Analytical epidemiology is the ascertainment of whether a particular exposure, such as a physical, chemical, or biological agent, and a specific cancer or other disease are unrelated (independent) or associated.
Etiology	The study of the cause of disease.
False negative	In analyzing the validity of a screening test for disease, those people who truly have the disease who are erroneously called "negative" by the test.
False positive	In analyzing the validity of screening tests for disease, those people who do not have the disease who are erroneously called "positive" by the test.
Historical cohort	A study using a cohort defined in the past.
Incidence	The number of new events or cases of disease that occur in a defined population at risk within a specified period of time. Incidence rates can be used to evaluate the changing patterns of disease frequency within a population and to assess the effectiveness of screening programs and treatment modalities on disease development.
Intervention	A study employed to test the efficacy of a preventive or therapeutic measure to generate knowledge about the etiology and natural history of a disease so as to formulate strategies for its prevention. Clinical trials are intervention studies that focus on the individual. They compare the outcomes in a group of patients treated with the test treatment with those observed in a comparable group of patients receiving a control or placebo treatment, where patients in both groups are enrolled, treated, and followed over the same time period. Community interventions focus on the group or community and evaluate the benefits of new policies and programs, determining which have an effect on the health of those who receive the intervention and which do not.
Nested case-control	A study where a series of cases are identified using a case-control approach within the confines of a well-defined cohort study. The case group consists of a representative sample of individuals, with the disease of interest occurring in the defined cohort over a specified follow-up period.
Population	The number of persons in a defined group who are capable of developing the disease. Can also refer to the general population; a population specifically defined by geographic boundaries, physical or social characteristics, or risk; the sampling population; and the study population.

(continued)

having similar and relevant characteristics. In this instance, the control group can be regarded as a reasonably representative sample of the case reference population. The selection of an appropriate control group represents the major challenge with case-control studies and often serves as the source of selection bias introduced into the study.[6]

The information gained from case-control studies does not establish a causal relationship between the disease and the exposure, but it does explore the concurrent association between the two. If the strength of this association is significant and supported by other studies, it can be used to justify the use of larger cohort studies or clinical trials that can investigate causative relationships.

When conducting a case-control study, be aware that cases and controls may differ in characteristics and exposures aside from the ones that have been

Table 3-1   Glossary of Epidemiological Terms (continued)

Power	The probability that a study will have the statistical strength to detect relationships that exist between exposures and disease. The power of a study can be maximized by controlling for factors such as sample sizes, measurement error, and bias.
Prevalence	The number of new and existing cases of a given disease or condition in a defined population within a specified period of time. Point prevalence refers to prevalence at one point in time. Period prevalence refers to prevalence between two points in time. Prevalence rates can be used to compare disease frequencies across populations and to assess the magnitude of effect of certain diseases on the health status of a population.
Rates and ratios	Calculations used to compare the frequencies of diseases in a population. Commonly used rates and ratios are given in Table 3-2, which lists the rate names. The numerator and denominator values and the population factor are used to express the rate in a standard format.
Risk measures	Attributable risk is the arithmetic or absolute difference between the exposed group and the nonexposed group in terms of incidence rates or death rates. It estimates the number of disease cases that can be attributed to or explained by the exposure (e.g., the majority of lung cancer cases can be attributed to exposure to cigarette smoking).
	Relative risk (RR) is a ratio comparing the rates of a disease among the exposed group and the nonexposed group that serves as a measure of the association between the disease and the exposure. The RR is generally used in cohort studies. The formula for calculating it is $$\frac{a(a+b)}{c \neq (c+d)}$$
	The odds ratio (OR) approximates the relative risk by comparing the rates of disease among the exposed and nonexposed groups. The OR is generally used in case-control studies with smaller sample sizes. The formula for calculating it is $$\frac{ad}{cb}$$
	Both the RR and OR are expressed as ratios (e.g., an OR of 1.0 means the rate of disease among the exposed group equals the rate among the nonexposed group).
Sensitivity	Measures the probability that a screening test will correctly classify an individual as positive for a disease when he or she actually does have the disease.
Specificity	Measures the probability that a screening test will correctly classify an individual as negative for a disease when he or she actually does not have the disease.
Spurious	As applied to associations between exposures, a false relationship produced by methodological errors or confounding variables.
Validity	Internal validity is the extent to which the subjects in an epidemiological study are truly comparable with respect to general characteristics (e.g., if most of the cases are from an urban setting and the controls are mainly from a rural setting, the two groups are not comparable, and evaluation of the exposure–disease relationship may be affected by these differences). Internal validity is essential for the interpretability and reliability of a study.
	External validity, or generalizability, is the extent to which the study population can be compared with a larger population (e.g., the general population). External validity must be assessed before study results can be applied to a broader population (e.g., a study that uses as its population a specific profession, such as nurses, may yield results that are not relevant to all women in the general population; while the study may have strong internal validity, the participating nurses may not be representative of the women in the general population or in the nursing profession).

Adapted from Reid M: Cancer control and epidemiology, in Yarbro CH, Frogge MH, Goodman M, et al (eds): *Cancer Nursing Principles and Practice* (ed 5). Sudbury, MA, Jones and Bartlett, 2000, pp 60–82.[4]

targeted for the study. Suppose we were interested in conducting a case-control study to determine whether lung cancer was linked to cigarette smoking. With this study design, we would start with the disease outcome (e.g., lung cancer) and retrospectively examine the extent of smoking among cases and controls. Age, in this instance, is related to length of smoking history as well as to cancer of the lung. The confounding effect of age can be avoided by selecting cases and controls of the same age group or matching the two groups for age.[7]

Matching is the process of selecting controls for factors beyond the targeted factors for the study so that the controls are similar to the cases in certain characteristics, such as age, race, sex, social economic status (SES), menopausal status, and occupation.[5] Two matching techniques are used in epidemiologic research: frequency matching and individual matching. In frequency matching, the proportion of controls with a certain characteristic is identical to the proportion of cases with the same characteristic. In individual

Table 3-2 Rates and Ratios Commonly Used in Epidemiology

Rate Name	Rate Description	Population Factor
Crude birth rate	Number of live births / Average or midyear population	per 1000
Fertility rate	Number of live births / 15- to 41-year-old women at midyear	per 1000
Crude mortality rate	Total number of deaths / Total population at midyear	per 1000
Age-specific mortality rate	Deaths in specific age group / Midyear population in age group	per 100,000
Cause-specific mortality rate	Deaths from a specific cause / Total midyear population	per 100,000
Infant mortality rate	Deaths of children younger than 1 year of age / Number of live births	per 1000
Neonatal mortality rate	Deaths in infants younger than 28 days / Number of live births	per 1000
Case fatality rate	Number of deaths from a disease in a given period of follow-up / Number of diagnosed cases of disease at start of follow-up period	per 1000
Proportional mortality rate	Number of deaths from a given cause / Number of deaths from all causes	per 1000
Morbidity rate	Number of cases of the disease that develop in a given period / Total population at midperiod	per 100,000

Adapted from Reid M: Cancer control and epidemiology, in Yarbro CH, Frogge MH, Goodman M, et al (eds): *Cancer Nursing Principles and Practice* (ed 5). Sudbury, MA, Jones and Bartlett, 2000, pp 60–82.[4]

matching (also known as matched pairs), a control is selected for each case that is similar to the case in terms of the specific variable or variable of concern. If the first case enrolled in our study is a 50-year-old post-menopausal white woman, we would therefore seek a 50-year-old white postmenopausal control. The advantage of matching and analyzing the data for pairs of subjects is that fewer subjects are required in each group to discern a relationship between the exposure and the disease. Matching enhances the ability to sub-stantiate a true association between exposure and disease outcome. It is useful when small numbers of case subjects with the disease are available for study and when efficiency is a major issue. Matching also provides a means for controlling potential confounding introduced by the selection of the control group.

The following example describes a case-control study that used matching to examine cervical cancer, a disease where little is known about the actual causes, though many suspected risk factors have been studied.
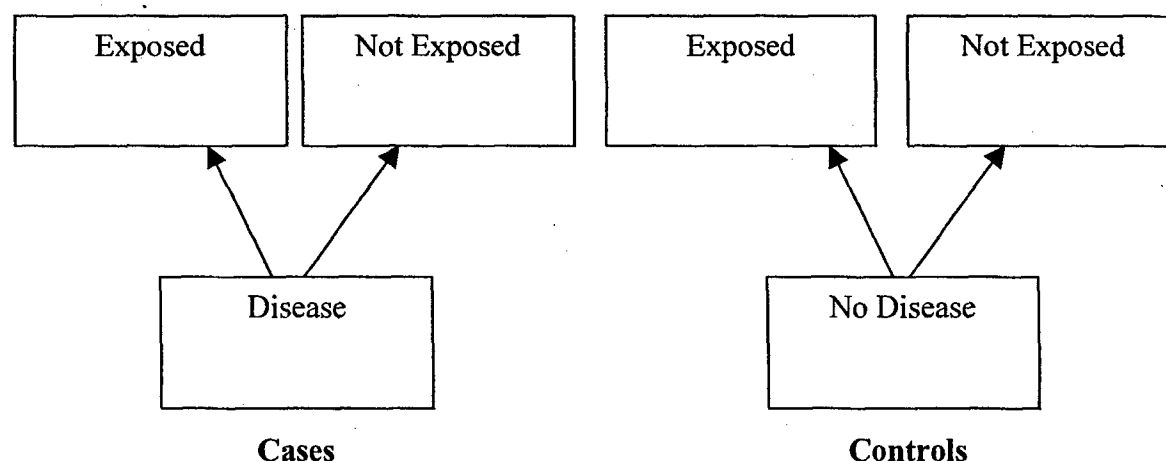
Figure 3-1 Design of a case-control study. Reprinted from *Epidemiology*, Gordis, L, pp. 118–119, Copyright 1996, with permission from Elsevier.[5]

```
                    ┌──────────────┐
                    │   Defined    │
                    │  Population  │
                    └──────────────┘
                           │
                    Nonrandomized
                     ╱           ╲
         ┌──────────────┐   ┌──────────────┐
         │   Exposed    │   │ Non-exposed  │
         └──────────────┘   └──────────────┘
           ╱        ╲         ╱        ╲
  ┌─────────┐  ┌──────────┐ ┌─────────┐ ┌──────────┐
  │ Disease │  │No Disease│ │ Disease │ │No Disease│
  └─────────┘  └──────────┘ └─────────┘ └──────────┘
```
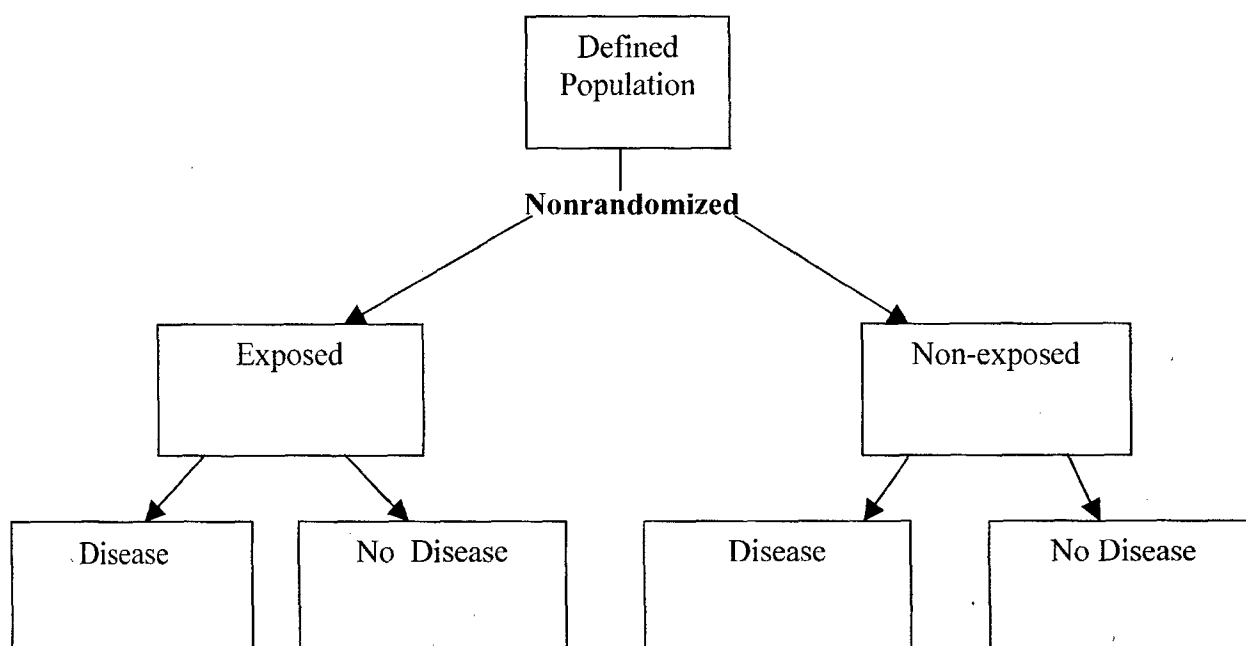
Figure 3-2   Design of a concurrent cohort study. Reprinted from *Epidemiology*, Gordis, L, pp. 118–119, Copyright 1996, with permission from Elsevier.[5]

The cases were 480 patients with invasive cervical cancer diagnosed at 24 different hospitals. Patients were between 20 and 74 years. A total of 797 controls were identified and matched by telephone exchange, race, and five-year age group. Data were collected through home interviews that included questions on hypothetical cervical cancer risk factors such as smoking, sexual behavior, pregnancy history, menstrual history, oral contraceptive use, medical history, diet, marital status, and family history. The results illustrated that compared to never-smokers, the women who smoked 30 or more cigarettes per day were 3.2 times more likely to have cervical cancer.[8]

Two immediate problems arise with matching. First, if an attempt is made to match too many characteristics, it may prove difficult or impossible to identify an appropriate control. Second, once cases and controls have been matched according to a given characteristic, that characteristic cannot be studied in relation to disease. Caution is advised on matching on any variable that may be of interest for exploring in a study.

Cohort studies

A cohort study seeks to investigate whether the incidence of an event is related to a suspected exposure. That is, a cohort study is an incidence study. It starts with a group of subjects who are at risk for developing a disease, yet are free of the disease at the beginning of the study, as shown in Figure 3-2.[5] Cohort studies can be envisioned as going from cause to effect. The expo-

sure of interest is determined for each member of the cohort, and the group is followed to document incidence of disease in the exposed and nonexposed members.

Cohort studies can be prospective, retrospective, or ambidirectional. Cohort studies are considered *prospective* or *concurrent* when the cohort is assembled at the present time and the subjects are followed concurrently through calendar time until the point at which the disease does or does not develop. The disadvantages of prospective studies relate to the amount of time needed to conduct them to determine whether the outcome of interest has developed and their usually exorbitant costs.

The Nurses Health Study is one of the most prominent examples of a prospective cohort study.[9-11] Nurses between the ages of 25 and 42 years old, living in one of 14 selected states, were enrolled in the study when they responded to a questionnaire about their medical histories and lifestyles in 1976. Follow-up questionnaires were sent biennially to update information on risk factors and medical events. All eligible nurses were studied for weight gain, hypertension, dietary intake, reproductive behaviors, menopausal status, family history, hormone replacement therapy (HRT), physical activity, medical history, smoking status, and alcohol consumption. Blood samples have allowed researchers to explore biomarkers and genetic factors.[10] This study is now in its third wave of data collection and has addressed several hypotheses germane to women's health and female cancers, including the

association of estrogens, tubal ligation, folate intake, menopausal status, and weight gain with cancer risk.

An alternative approach to the cohort study design is nonconcurrent cohorts, also known as *historical* or *retrospective cohort studies.* A previously defined cohort is identified and assembled in the past on the basis of existing records, and disease outcome (development or no development of disease) is ascertained at the time the study is begun (Figure 3-3). Nonconcurrent studies are notably less expensive and can be implemented more expeditiously than concurrent studies. Their main disadvantage is reliance on available information; consequently, the quality of exposure or outcome data is sometimes less than ideal for fulfilling the study objectives. Many occupational cohort studies are conducted retrospectively.

Case-control studies within a cohort study are known as *ambidirectional studies* or *nested case-control studies,*[11] because they combine some of the features and advantages of both cohort and case-control designs. The selection of participants is carried out using a case-control approach, as shown in Figure 3-4. A nested case-control design starts with a previously established cohort and continues subject follow-up into the future. Ambidirectional designs are being used increasingly for cost-efficiency reasons when analysis of all cohort members requires substantial resources.[11]

Clinical trials and intervention studies

A clinical trial or intervention study is a planned experiment testing medical treatments. This type of study is designed to assess the efficacy of a treatment by comparing outcomes in a group of patients who received the test treatment with outcomes observed in a comparable group of patients who receive a control treatment. Both groups of patients are enrolled, treated, and followed over the same time period.[12]

Once clinical trial patients have been screened for eligibility, they are randomly assigned to one of the study groups. There is an intervention or a treatment group and a control arm of the trial, which receives the placebo or the current therapy. A randomized clinical trial may, for example, randomly assign a group of cancer patients to a particular drug regimen and assign a similar group of cancer patients to a course of not receiving the drug. The two groups are monitored over the duration of the study, with researchers comparing the groups' survival or cure ratio of cancer.

To preserve the objectivity of the data gathered in clinical trials, the blinding approach is used. Participants are blinded as to which group assignment they will get, either the treatment or the control group. This technique prevents attrition when subjects who learn that they have been randomized to the placebo
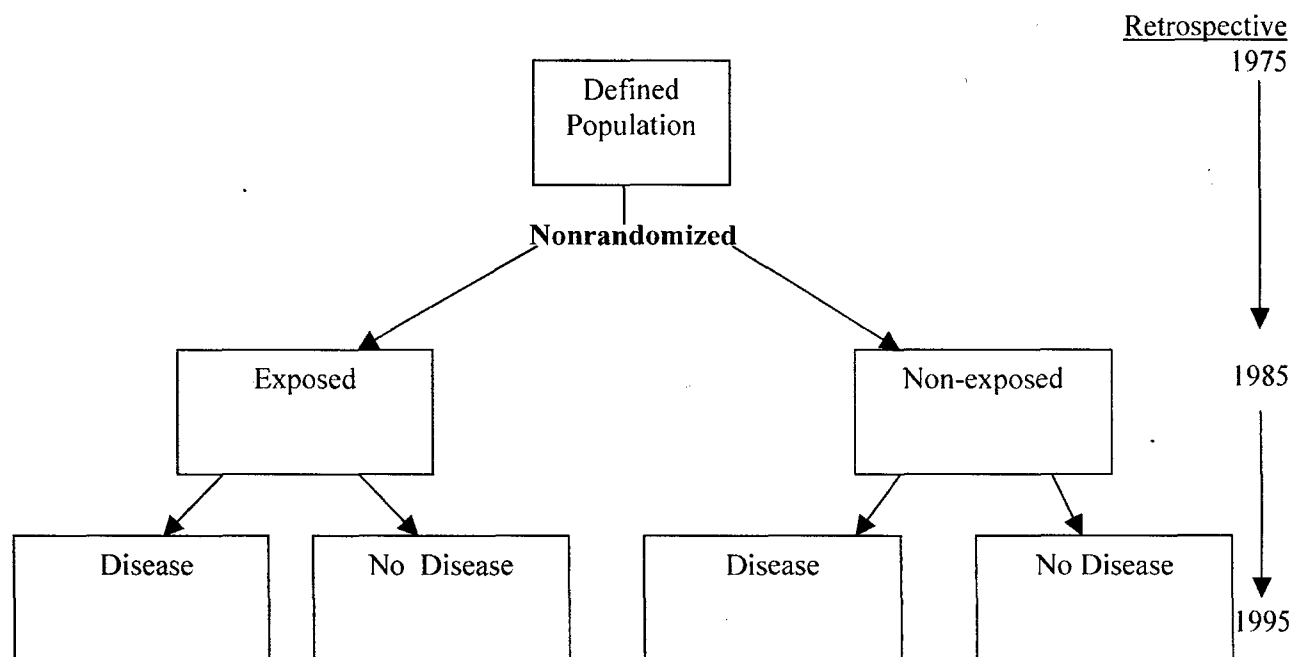
Figure 3-3 Design of a nonconcurrent cohort study. Reprinted from *Epidemiology*, Gordis, L, pp. 118–119, Copyright 1996, with permission from Elsevier.[5]
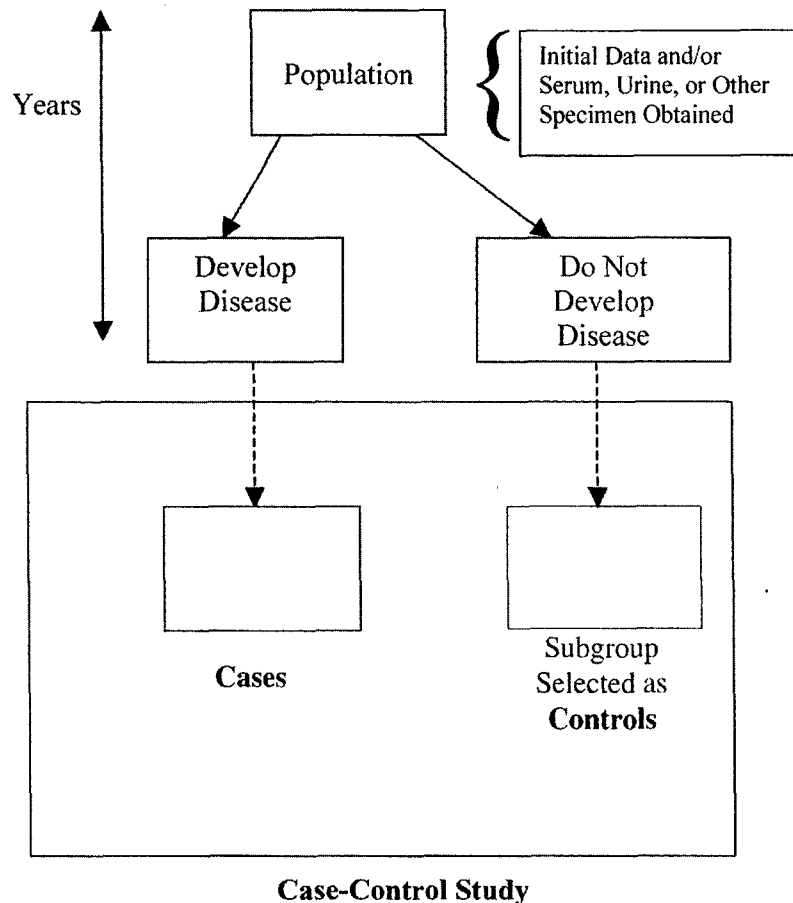
Figure 3-4   Design of a nested case-control study. Reprinted from *Epidemiology*, Gordis, L, pp. 118–119, Copyright 1996, with permission from Elsevier.[5]

arm of the trials subsequently drop out. Additionally, the investigator can be blinded as to the subject's group assignment, creating a double-blind design. A double-blind design protects against the investigator becoming biased as to the trial's outcome, particularly if a drug manufacturer is financing the trial.[2]

A major benefit of a double-blind, placebo-controlled clinical trial is that the random assignment of treatment groups helps to distribute potential confounding variables evenly between the two groups, thereby minimizing their effects on the measurement of the association between the exposure and the disease. If this control of confounding is successful and the primary difference between the two treatment groups is the intervention, then a clinical trial can definitively evaluate the efficacy of the intervention.

An example of a clinical trial is the Physicians' Health Study,[13] which randomized 22,071 licensed physicians into an expanded design to test the effectiveness of aspirin on decreasing the rates of heart attacks and the effect of beta-carotene on inhibiting the development of cancer. This study was defined as having a multifactorial design. After five years, the aspirin arm of the trial was stopped because a significantly lower risk of heart attack was observed among the subjects receiving aspirin. The beta-carotene arm of the trial was discontinued in December 1995; no effect of beta-carotene was observed on cancer incidence.[14]

A major limitation of the clinical trial design is that several years of subject follow-up may be required before significant changes in the rate of disease development are observed among treatment groups. The length of follow-up will depend on several factors, including the strength of the effect the treatment has on the risk of the disease. Long-term studies raise patient management issues, such as maintaining active participation of subjects, monitoring subject deaths and adverse events, and tracking subjects lost to follow-up. These factors, if unevenly distributed among the treatment groups, may confound the results of the project.

Experimental studies

Experimental studies maintain the greatest control over the research setting. Random allocation is used to assign subjects either to receive the treatment or to not receive it or to be assigned to either the exposed or the

nonexposed group. Once substantial and consistent evidence has accumulated from experimental studies, other study designs may be employed to further demonstrate the feasibility of large-scale population interventions.

The randomized control trial dominates experimental research in laboratory animals. Performing such experiments on humans does have its drawbacks, however, the most important of which are ethical. It is obviously not acceptable to expose humans intentionally to a potential carcinogenic agent in an attempt to ascertain cancer causation.[11]

Ecological studies

Ecological studies in epidemiology occupy an intermediate position between descriptive and analytical investigations, in that they share characteristics with descriptive studies but serve etiologic objectives.[11] The exposure and disease under investigation in ecologic studies are not ascertained for specific individuals but rather across groups and whole populations. The unit of measure is the geographic region, not individuals within that region.[15] When an exposure is fairly common, such as smoking, sunlight, or fat consumption, ecological studies can elucidate the possible effects of these exposures. For example, skin melanoma is more common in geographic latitudes with more sunshine exposure, and countries with higher per capita intakes of dietary fat tend to be the same countries with high rates of breast cancer mortality.[16] The caveat of using ecological studies to prove disease causality is the phenomenon of ecological fallacy: "the bias that occurs because an association observed between variables on an aggregate level does not necessarily represent the association that exists at an individual level."[17]

Despite their limitations, ecological studies do have merit within epidemiologic research. They are quick, simple to conduct, and inexpensive. When little is known about the association between an exposure and disease, an ecologic study is a reasonable place to start generating hypotheses.[2]

Cross-sectional studies

Cross-sectional studies allow the investigator to study the relationship between an exposure (e.g., electromagnetic fields) and a disease outcome (e.g., leukemia) by surveying a population for each participant, and determining the exposure and disease outcome simultaneously.[7] The unit of analysis in cross-sectional studies is the individual. Cross-sectional studies are referred to as "snapshot" studies because they provide a one-time view of a population's rate of existing (prevalent) cases of the disease, the degree of exposure, and other demographic characteristics of interest at a single hypothetical point in time. While cross-sectional studies cannot establish a causal relationship between the exposure and the disease, they do provide descriptive statistics for the population and are often used as the preliminary step in establishing disease or exposure status in cohort studies.

Defining the Disease

Defining the disease in epidemiologic studies is the penultimate task in including and excluding the appropriate subjects in a study population. Disease may be defined by review of medical records, pathologic results, blood test results, physical exam, histologic characteristics, or results from a psychological battery of tests. To increase the rigor of this step, two different medical professionals, each unaware of the other's findings, should confirm disease status. Clearly stating disease definition guidelines at the outset can prevent enrolling subjects who are actually ineligible for the study. Once a disease status is confirmed for each subject, he or she is eligible for study enrollment.

Eligibility and Exclusionary Criteria

Study eligibility is determined by a set of criteria to gather a population of subjects with a sufficient prevalence of disease to test the hypothesis efficiently. Eligibility criteria in cancer research are typically age ranges, gender-specific factors, race, disease stage, life expectancy, absence of other cancers except nonbasal cell carcinoma, exposure to certain drugs, treatments, and current health status. A strict definition of exclusionary criteria should also be stated as part of the study subject screening process. Exclusionary criteria may involve previous medical history, inability to provide informed consent due to mental competency, a permanent address if the study design is conducted via the telephone or mail, and proficiency in a particular language if the study materials are written and administered in one language.

Interrelated with the definition of disease is how the disease was contracted, or exposure to which factors that lead to a disease state. An exposure in epidemiology comprises the subject's contact with the variable of interest, which may influence the development or improvement in disease status. Exposures run the gamut from microenvironmental exposures on an individual level, such as nutrients, medications, physical activity, and genes, to macroenvironmental exposures, such as air pollution and environmental conditions that affect an entire community.[11] In epidemiologic research, exposures are measured by their frequency

and duration as well as their ability to synergistically react with one another.

Dose refers to a standardized, measured amount of exposure issued (e.g., standard milligrams, as in the case of drugs; gray [Gy] for radiation; number of packs of cigarettes per year; hours of exercise; drinks of alcohol per day). It is imperative to assess whether the dose has remained constant throughout the exposure or whether certain variables or conditions have affected the dose over time. The likelihood of an association between an exposure and disease being causal is stronger if a more intense "dose" of the exposure produces higher rates of disease.

Defining the Population

In addition to defining the type of study design appropriate for testing a research hypothesis and the disease/exposure, the source population for study subjects and the actual study population must be defined. This process clarifies to whom the research results can be generalized (external validity), whether the study population represents the total population and the source population, and what the overall characteristics of eligible subjects are.

The source population for the study is the larger group or population from which the study subjects are recruited. It might include, for instance, residents in a certain city or neighborhood, university students, or all patients attending a particular hospital. The source population is usually a subgroup of the total population.

The study population is the group of subjects actually recruited into the project from the source population. Recruitment into the study population, based on the defined eligibility and exclusionary criteria, is planned to access all potential subjects within the source population. It is important to review the types of subjects who were part of the source population but who were not eligible or not approached for recruitment. For example, if subjects were recruited from phone interviews, we could safely conclude that only subjects with telephones were eligible. Because the presence of a telephone in the household might be related to socioeconomic status (SES), it is possible that the study population might be biased toward subjects with a higher SES. The relationship of SES to the disease may be impossible to evaluate and may affect the results of the study.

Statistical Plan

Epidemiologic research measures disease outcomes in rates and ratios, frequencies, and distributions. Aside from these descriptive statistics, inferential statistics can be used to infer whether the exposure disease relationship that is observed in the study population is applicable to a larger population. This premise is called *external validity* or *generalizability*. Additional factors play a role in establishing causation between exposure to risk factors and disease outcome.

Potential Sources of Bias and Confounding Variables

To reasonably assert an uncompromised relationship between exposure and disease, we must account for any bias that exists in an epidemiologic study design. The most common forms of bias in a case-control study are (1) including noncases in the case series of subjects, (2) a systematic error in data collection, (3) an inordinate amount of random error in the collection of data, and (4) inappropriate analysis of data. These types of bias result in a mistaken estimate of an exposure's effect on the risk of disease.[18]

Two primary forms of bias are encountered in epidemiologic studies: selection bias and recall bias. *Selection bias* arises when the relationship between exposure and disease is different for those who participate in the study and those who would be theoretically eligible for the study but did not participate.[19] For instance, the healthy worker effect may occur in occupational cohort studies. Only employed individuals are eligible for such a study, but workers are relatively healthy people who are able to maintain employment.[2] The characteristics of these individuals are, therefore, not generalizable to the overall population. *Recall bias* results from inaccurate recall of past exposures. It is especially a concern in the context of case-control studies when cases and controls are queried for exposures in the past. Bias on the selection of subjects or the study results can lead to a spurious, or unrelated association as contributing to the exposure–disease relationship factor.

Another concern in epidemiologic studies is confounding variables. Confounding variables prevent study groups from being comparable. For instance, if a case-control study shows an association between alcohol intake and lung cancer, we must investigate whether a third factor might exist in the causal pathway of a lung cancer outcome, one that was not originally stated in the study hypothesis. Smoking is another complementary risk factor associated with drinking alcohol. Smoking in this case is a confounder. Although we were interested in the causal relationship between alcohol consumption and lung cancer, smoking is a known risk for lung cancer; it is associated with alcohol intake but is not a result of drinking alcohol.[5]

When planning an epidemiologic study, consult a biostatistician or epidemiologist to help design the research protocol. Sample a large enough study group to have the ability to draw causal inferences for the general population and to perform a rigorous statistical analysis. A variety of techniques can be used to control for bias and confounding variables in epidemiologic studies. Randomization, matching, and statistical analyses stratifying or controlling for confounding variables are commonly used methods. In summary, retain experienced researchers to minimize potential errors at the data-set phase of the study design. Various statistical[18,19] and epidemologic texts[2,5,7,11,20] are excellent resources for fundamentals of epidemiologic and statistical practice.

Data Sources

There are several types of data sources in epidemiology from which to glean information on cancer research and data sets, as shown in Table 3-3 — computerized bibliographic databases, repositories of vital statistics, disease registries, hospital clinic data, and survey research on the general population. When utilizing epidemiologic data from these sources, be cognizant of the availability and completeness of the data. Also, obtain the necessary permission to access the data. Legislation has been enacted to protect identifying information on medical patients. Additionally, a given data set for a target population may not be representative of the general population and lack generalizability.

Causes of Cancer

Tobacco

The causal relationship between tobacco use and various forms of cancer has been primarily derived from epidemiologic research. Epidemiologic studies of tobacco have relied on mostly self-reported smoking histories. Unlike with dietary patterns, subjects participating in epidemiologic research are unlikely to seriously misreport their smoking habits owing to faulty recall.[21]

Active tobacco use has been linked to many cancer types: lung; lower urinary tract, including the renal pelvis and bladder; upper aero-digestive tract, including the oral cavity, pharynx, and esophagus; and pancreas. Smoking tobacco can also cause cancer of the nasal cavity, paranasal sinuses, and nasopharynx; stomach; liver; kidney; cervix uteri; adenocarcinoma of the esophagus; and myeloid leukemia. Cancer can be caused by smoking cigarettes, pipes, cigars, or bidis (a

Table 3-3 A Selection of Internet Addresses for Sites Dealing with Epidemiology and Cancer

American Cancer Society	www.cancer.org
American Lung Association	www.lungusa.org/
International Agency for Research on Cancer	www.iarc.fr
National Cancer Institute	cancer.gov/cancerinformation
National Center for Health Statistics	www.cdc.gov/nchs/
Office of Minority Health	www.omhrc.gov/OMH/sidebar/datastats.htm
Oncolink	www.oncolink.org/
SEER Cancer Statistics	seer.cancer.gov/
U.S. Census Bureau	www.census.gov
Virtual Library: Epidemiology (Biosciences and Medicine), University of California, San Francisco	www.epibiostat.ucsf.edu/epidem/epidem.html
World Health Organization	www.who.ch/whois/whois.htm

small amount of tobacco wrapped in the leaf of another plant, commonly used in South Asia).[22,23]

Passive Smoking

The carcinogenic effects of environmental tobacco smoke (ETS) or passive smoking on human lung tissues have been a hotly debated issue during the past decade. ETS can be defined as sidestream smoke and mainstream smoke exhaled by active smokers. Both forms of ETS contain about 40 different chemicals that are suspected or proven carcinogens.[21] In 1992, the U.S. Environmental Protection Agency (EPA) published a report that classified ETS as a group A carcinogen (known human carcinogen). Approximately 90% of the epidemiologic studies on ETS focused on non-smoking women married to smokers. The annual number of cases attributable to spousal ETS is on the order of 50 in men and more than 500 in women. The corresponding estimates for ETS exposure at the workplace are about 200 cases among men and 270 cases among women.[24] Estimates from the EPA for the U.S. population, which considered spousal and background sources of ETS, predicted in 1930 cases among women and 1130 cases among men. The evidence for a causal association between ETS exposure and cancer in organs other than the lung is inconclusive.[8,25] The overall estimate of risk associated with ever being exposed to ETS and lung cancer is a 1.2 greater risk for lung cancer in married women based on spousal smoking.[26] Being exposed to ETS in the workplace also

conveys a 1.2 greater risk of developing lung cancer. Although results from studies of passive smoking and breast cancer risk have been inconclusive, new findings on active smoking status and breast cancer reveal a 1.3-fold greater risk of breast cancer in smokers compared to women who have never smoked and were not exposed to passive smoking.[27]

Individuals who use smokeless tobacco, such as chewing tobacco and snuff, experience an increased risk of oral cancer. The evidence to date from epidemiologic studies indicates no relationship between smokeless tobacco and bladder cancer, but suggestive evidence links smokeless tobacco use to prostate cancer risk. Hemoglobin adducts to carcinogens present in smokeless tobacco products are found in measurable levels in the blood of smokeless tobacco users, indicating that smokeless-tobacco–related carcinogens circulate throughout the body. This prompts a concern that smokeless tobacco may increase risks of other cancers as well.[28,29]

Diet

Diet may be of great importance in cancer prevention. It has been proposed as a contributing factor in 20%–70% of cancer deaths[30,31] and is considered a modifiable risk factor. Interest and research in the role of diet in cancer have flourished in recent years, with many micronutrients (vitamins and minerals) and some macronutrients (proteins, fats, carbohydrates) being investigated for adverse or protective effects against cancer, in both human and animal studies.[32,33] The impetus for many of these studies came from the results of ecological studies. For example, a high correlation was found between national per capita daily meat consumption and country-specific colon cancer incidence rates.[34]

Cancer and macronutrients

Fat intake. Historically, results from case-control and cohort studies generally have supported high fat intake as a risk factor for colon cancer.[35–38] The role of fat in colon cancer is supported by both the rapid change in incidence with dietary change and the potential relationship of fat consumption to bile acids, which are known to be mutagenic.

Nevertheless, the relationship between fat and colon cancer has yet to be firmly established. Ecological studies that use data from many countries show a strong positive relationship between per capita fat intake and breast cancer mortality rates.[34] At the same time, case-control and cohort studies give conflicting results. In a combined analysis of 12 case-control studies of dietary factors and breast cancer, an association

was found between high fat intake and breast cancer in postmenopausal women.[39] However, the analysis of data from seven cohort studies in four countries showed no evidence of a positive association between total dietary fat and the risk of breast cancer.[40] Two of the largest cohort studies, the Nurses Health Study[41] and the Iowa Women's Study,[42] showed no relationship between dietary fat intake and breast cancer risk, although some researchers suggest that this outcome may be because the range of fat intake in such studies was too small. Current dietary recommendations are for women to reduce fat intake to less than 30% of calories. In Willett's study, the range of fat intake was 32%–44% of calories.[41] The notion that fat intake may be related to breast cancer has persisted, but there has been an inability to provide individual — as opposed to national — statistics relating breast cancer to fat intake. This result has led to a wide acceptance that the relationship is not to fat but rather to total calories and especially to total calories consumed early in life.

Cancer, micronutrients, supplements, and intake of fruits and vegetables

One of the most consistent dietary findings in analytic epidemiological studies with regard to cancer is the protective effect of fruits and vegetables.[43] Which particular nutrient, non-nutrient, or combination in fruits and vegetables offers protection against cancer remains under investigation. The roles of several micronutrients in cancer prevention, including the carotenoid beta-carotene, vitamin A, vitamin E, and selenium, have been extensively investigated. Relatively high levels of these four micronutrients have been found to be associated with lower cancer risk in many studies, although again not all study results are in agreement.[31,33,44–47]

Some have speculated that the antioxidant effect of vitamin C might play a preventive role against cancer, but this relationship has not been well established.[48,49] Various studies strongly suggest that folate has effectiveness in cancer prevention.[50] Folate is critical for both DNA synthesis and DNA methylation, and various mechanisms have been hypothesized through which folate might influence carcinogenesis. Dietary and supplemental folate appear to be protective and reduce the risk of pancreatic,[51] breast,[52] and colorectal cancer.[53] The optimal dose of folate to minimize colorectal cancer has not been established. Preliminary evidence based on pooled results from nine cohort studies suggest that intakes of approximately 400–500 μg/d may be required to minimize risk.[53]

The antioxidant effects of lycopene may explain the reduction in gastrointestinal tract, breast, and cervical cancer incidence in some populations.[54] Various observational studies have explored the role of

lycopene, a carotenoid derived from tomato products like pizza, spaghetti sauce, ketchup, and salsa, in conjuction with gastrointestinal, breast, prostate, lung, and cervical cancer incidence. The mechanisms for the cancer-preventing actions of carotenoids may involve antioxidant activity, induction of detoxifying enzymes, and inhibition of cellular proliferation.[54-56]

There has been a growing interest in the preventive and therapeutic effects of phytoestrogens on various hormone-responsive cancers, such as breast, endometrial, ovarian, and prostate cancer. The phytoestrogens are compounds found in plants such as soy. The isoflavones, which show structural similarity to mammalians estrogens,[57] are considered to be responsible for providing the anticancer benefit. Isoflavones are present in large amounts in soybeans and soy products such as miso and tofu, kudzu root, peanuts, and lentils.[58] Their chemopreventive properties result from soy isoflavones possessing estrogenic activity, competing with estradiol for the estrogen receptor complex, and inhibiting hormone response in cancer cells and tumor growth.[59]

Fiber intake. A majority of studies of differing epidemiological designs support the hypothesis that high fiber intake is protective for colon cancer.[60] The well-documented relationship between meat consumption and colon cancer likely reflects the role played by animal fat consumption. The role of fiber in colon cancer has repeatedly been postulated to relate to altered transit time, altered bacterial flora in the colon, and altered exposure of the colonic mucosa to potentially carcinogenic bacterially modified bile acids. Epidemiological studies have suggested an inverse relationship between dietary fiber and colon cancer, and animal studies suggest that the type of fiber may be important,[61] although not all results are supportive of this position. Vegetables as well as cereals are sources of fiber. In studies where the source of fiber has been examined, fiber from vegetables appears protective against colon cancer, whereas the data for cereal fiber are less supportive of a protective effect. According to the hypothesized mechanism, fiber affects the bile acid content of the aqueous portion of stool. These differing results may be due to the difference in composition of fiber in cereals and vegetables or to the lack of a large range in cereal fiber intake, or they may indicate that some other chemical or nutrient in vegetables is protective against colon cancer.[60,62]

Calcium intake. A protective role for high calcium intake against colon cancer has been reported in several studies[63-65] but not in all.[36,66,67] Data from supportive studies suggest that to reduce the risk of colon cancer, calcium intake should be 1500 mg for females and 1800 mg for males.[68] Calcium may inhibit colorectal carcinogenesis because of its ability to bind toxic bile acids, thereby rendering them inert, or by direct effects on the cell cycle.[69] The role of calcium in colon cancer etiology is linked to vitamin D, as this micronutrient mediates intestinal calcium absorption.[70]

Case-control and cohort studies of diet and cancer present some measurement problems:

1. The distribution of dietary components among individual foods varies greatly. The interactive roles of dietary components are not completely understood, particularly when several components are present in individual foods.[71]
2. Recall bias may be present if dietary assessment is conducted after the presentation of the disease, as in a case-control study. In essence, individuals' recall of their past diet may be affected by their knowledge that they have the disease.[72] To avoid the problems associated with self-reported dietary intake methods, direct assessment of some micronutrients has been developed, involving measuring serum micronutrient levels. Issues regarding measurement of micronutrients through biospecimens are discussed later in this chapter.

Alcohol

Alcoholic beverages consist primarily of ethanol, water, and volatile and nonvolatile compounds. Numerous additives are also used in the production of alcoholic beverages, such as hops, synthetic flavor enhancers, preservatives, and trace elements.[73] Certain contaminants with proven mutagenic and carcinogenic properties have been detected in alcoholic beverages, such as N-nitrosamines, asbestos, arsenic compounds, and pesticides. Moderate to heavy alcohol use has been linked to cancers of the oral cavity, esophagus, larynx, bladder, and liver.[73] The association of alcohol consumption with cancers of the stomach, colon, and pancreas is less well established. Rectal cancer is the exception, for it appears to be associated specifically with beer consumption.[74] Nitrosamines that are found in beer have been suggested as a possible cause of the association between rectal cancer and beer consumption.[75] A cohort study of men who were past drinkers or who reported drinking more than two drinks per day, were non-aspirin users, and had low levels of folate intake had more than a sevenfold greater risk of developing cancer of the distal colon.[76]

Studies focusing on the relationship between alcohol and breast cancer suggest a positive but weak association. Alcohol has been well documented as a risk factor in head and neck cancer and more recently has

been implicated in breast cancer,[77-81] although this observation remains controversial. A majority of the findings from epidemiologic studies have shown a moderately increased breast cancer risk among women who consume moderate to high levels of alcohol.[80-82] Both the level of alcohol consumption required to significantly increase breast cancer risk[81] and the age at which exposure to alcohol becomes important[82] are unclear.

Physical Activity

Increased physical activity consistently has been found to be protective against prostate cancer,[83] colon cancer,[61,84-86] and precancerous colon polyps.[86-88] A mounting body of evidence suggests that increased physical activity is protective against breast cancer.[88-90] Intense physical activity at the age of usual menarche may be especially important, because it can cause a delay in onset of menarche. Lifetime physical activity has been proven protective against breast cancer in a large case-control study of women from Shanghai, China. Graded reductions in breast cancer risk were noted in premenopausal and postmenopausal women who exercised in both adolescence and adulthood. These women who engaged in exercise for 16 years or longer periods reduced their risk of breast cancer by 43% and 64%, respectively.[91] The close interrelationship of physical activity with obesity and diet — two factors associated with many cancers — also makes its role in relation to cancer risk important to assess.[92]

Occupational Exposures

At least 10% of cancer deaths in the United States are attributable to workplace exposures. The reasons to study occupational causes of cancer are numerous.

1. An immense number of individuals spend large amounts of time at their jobs, and a growing repertoire of chemicals and physical factors are found in diverse workplaces of today.
2. Workers are generally exposed to much higher levels of potentially hazardous chemical and physical factors than individuals who are exposed to similar hazards in nonoccupational settings. This phenomenon of increasing environmental cancer rates in the occupational group should be heeded by the community at large, which is itself potentially at risk.
3. Cancer stemming from occupational exposures should be considered preventable. Evidence from epidemiological research confirming causal cancer agents should prompt the removal of the agents or

Table 3-4 Cancers Associated with Various Occupations or Occupational Exposures

Cancer	Substances or Processes
Lung	Arsenic, asbestos, bis(chloromethyl) ether, chromium compounds, coal gasification, mustard gas, nickel refining, foundry substances, radon, soots, tars, oils, acrylonitrile, beryllium, silica
Bladder	Aluminum production, auramine and magenta manufacture, rubber industry, leather industry, 4-aminobiphenyl, benzidine, naphthylamine
Nasal cavity and sinuses	Formaldehyde, isopropyl alcohol manufacture, mustard gas, nickel refining, leather dust, wood dust
Larynx	Asbestos, isopropyl alcohol, mustard gas
Pharynx	Formaldehyde, mustard gas
Mesothelioma	Asbestos
Lymphatic and hematopoietic system	Benzene, ethylene oxide, chlorophenols, chlorophenoxy, herbicides, x-radiation
Skin	Arsenic, coal tars, mineral oil
Soft-tissue sarcoma	Cholorophenols, chlorophenoxy herbicides
Liver	Arsenic, vinyl chloride

Adapted from Reid M: Cancer control and epidemiology, in Yarbro CH, Frogge MH, Goodman M, et al (eds): *Cancer Nursing Principles and Practice* (ed 5). Sudbury, MA, Jones and Bartlett, 2000, pp 60–82.[1]

adequate prevention of potential exposed workers.[92]

A summary of some occupational carcinogens that may cause cancer is found in Table 3-4.

Pollution

The relationship between drinking contaminated water and cancer has been established in Taiwan, where increased risk of lung cancer has been reported among people exposed to arsenic in drinking water. Trihalomethane, another more common pollutant of drinking water, may be linked to rectal and bladder cancer.[93] These compounds are produced by the action of chlorine on organic waste.

Assessing the association of air pollution with cancer in epidemiologic studies is more challenging. Specifically, it is complicated to measure past exposure to the relevant air pollution and the level of the exposure. Exposure to air pollution has been evaluated by counting the number of inhabitants in the community of residence near a major pollution source. These data

mainly take into account suspended particulates, sulfur oxides, and nitrogen oxides, which are agents not responsible for the carcinogenic effect of air pollution.[11]

One type of pollution that may indirectly increase cancer risk involves chlorofluorocarbons (CFCs), which are destroying the ozone layer in the stratosphere.[94] It is predicted that this destruction will allow more ultraviolet light to reach the earth's surface, thereby increasing the risk for nonmelanoma and melanoma skin cancer. Exposure to ultraviolet-B (UV-B) radiation has been implicated by laboratory and epidemiologic studies as a cause of two types of nonmelanoma skin cancers: squamous cell cancer and basal cell cancer. Studies predict that for every 1% increase in UV-B radiation, nonmelanoma skin cancer cases would increase by about 1%–3% each year during which the condition of the deteriorating ozone exists. Recent epidemiologic studies suggest that UV-B radiation plays an important role in causing malignant melanoma skin cancer; for each 1% change in UV-B intensity, the incidence of melanoma could increase from 0.5% to 1%.[95]

Viruses and Other Biological Agents

Viruses may contribute to approximately 15%–20% of human cancers throughout the world.[96] Table 3-5 identifies viruses associated with certain cancer sites. Viruses produce cancer in the host only after a substantial incubation or latency period. This latency period usually extends for years, hindering studies in linking the particular viral exposure with a particular cancer. When the initial infection with the candidate virus is subclinical, verification after clinical features emerge to establish the exact time of infection is compromised.

Several epidemiologists and experimental studies have established a casual role of Hepatitis B virus (HBV) and Hepatitis C virus (HCV) in the occurrence of hepatocellular carcinoma (HCC) and liver cancer.[96] Viruses are etiologically linked to approximately 20% of all malignancies worldwide.

The Epstein-Barr virus (EBV) has been linked with Burkitt's lymphoma and other B-cell lymphomas and nasopharyngeal cancer. EBV has also been implicated in the development of Hodgkin's disease.

The human T-cell lymphotropic virus (HTLV-1), which contributes to the development of human T-cell leukemias, has the highest incidence rate in Japan. This virus is primarily spread from males to females, through transmission in semen, and from mother to child, with breast milk being the likely vector. After a long latent period, adult T-cell leukemia/lymphoma (ATL) occurs in 1 per 1000 carriers per year, resulting in 2500–3000 cases per year worldwide and over half of the adult lymphoid malignancies in endemic areas.

Human immunodeficiency virus 1 (HIV-1) accounts for a significant cancer burden. Kaposi's sarcoma (KS) is a very rare tumor except after HIV-1 infection, when its incidence is greatly amplified, being magnified 70,000-fold in HIV-infected homosexual men. Human herpesvirus 8 (HHV-8), which is also known as Kaposi's sarcoma–associated virus (KSHV), is a necessary but not sufficient etiological factor in KS. The dramatic decline of KS incidence in recent years is due to the introduction of highly active antiretroviral therapy (HAART). B-cell non-Hodgkin's lymphoma occurs as the first acquired immunodeficiency syndrome–defining diagnosis in 3%–4% of HIV-infected patients. Hodgkin's lymphoma is also associated with HIV infection, albeit at a lower risk.

Human papillomaviruses (HPVs) are linked to invasive cervical cancer and anogenital cancers among HIV-infected patients. HPVs are DNA viruses that have been causally linked to cancers of the uterine cervix. Subtypes HPV-16, -18, -31 and -45 have been linked to cervical, penile, and anal cancers of the aerodigestive tract. HPV DNA is found in 93% of all invasive cervical cancers, with 50% of cases being infected with subtype 16.

Human retroviruses cause malignancy via direct effects as well as through interactions with other oncogenic herpesviruses and other viruses. In no case in humans, in contrast to animal and cell culture systems, has a viral infection directly produced a malignancy; in humans, cancer is a multistep process.[97]

Radiation

Ionizing radiation

The greatest source of exposure to ionizing radiation is background radiation in the environment. For U.S. residents, ionizing radiation from natural sources

Table 3-5 Cancer Types Associated with a Virus or Other Biologic Agent

Virus or Biological Agent	Cancer
Hepatitis B virus	Hepatocellular carcinoma
Human papillomavirus (types 16 and 18)	Cervical cancer
Epstein-Barr virus	Burkitt's lymphoma
Human T-cell lymphotrophic virus type I	Adult T-cell leukemia/lymphoma (ATLL)
Human immunodeficiency virus	Kaposi's sarcoma; non-Hodgkin's lymphoma
Schistosoma	Bladder cancer
Heliobacter pylori	Gastric cancer

accounts for approximately 82% of the total exposure from all sources. Background radiation includes naturally occurring cosmic rays and radiation from ground sources, such as uranium, radon, potassium, and other substances. It is problematic to conduct epidemiologic studies of potential cancer risk from naturally occurring background radiation due to the difficulty of measuring an individual's lifetime or cumulative exposure.[98,99]

From the standpoint of prevention, little more can be done than is already being done: minimizing exposure to man-made radiation hazards. It is notable, however, that stopping smoking has the greatest potential for preventing radiation-induced cancer of the lung, as radon exposure acts synergistically with tobacco smoke. Smokers who were exposed to radon while working as miners had ten times the incidence of lung cancer as did nonsmokers.[100]

In 1987, the International Agency for Research on Cancer designated radon, a radionuclide existing normally as an inert gas, as a human carcinogen. Radon exposure increases the risk of lung cancer among underground miners, and indoor radon exposure is the second leading cause of lung cancer in the United States. Radon itself does not directly cause lung cancer; rather, alpha particles from radon progeny directly damage target lung cells to cause cancer. In 1999, the EPA released a report on the health effects of indoor radiation, integrating findings from epidemiologic studies with evidence from animal experiments and other lines of laboratory investigation. The agency's report also considered the limited evidence on the synergistic effect of smoking and radon. According to this study, an estimated 157,400 people died of lung cancer due to radon in homes (from all causes, including smoking and radon exposure) in the United States. Of the 95,400 men who died of lung cancer, approximately 95% were probably ever-smokers; of the 62,000 women, about 90% were probably ever-smokers. Approximately 11,000 radon-related lung cancer deaths are estimated to have occurred in never-smokers.[101]

Radiation exposure found in medical treatments and diagnosis largely centers on the use of x-rays or irradiation treatment for various illnesses. The therapeutic radiation dosages given to cancer patients are among the highest levels received by humans. A large body of evidence indicates that organs can develop secondary cancers caused by radiation used in the treatment of a primary cancer. Treatment with ionizing radiation for the prevention of breast cancer is a controversial issue for this reason. In a risk-versus-benefits issue, healthy women are subjected to radiation to diagnose breast cancer at an early stage through mammography, a procedure with known carcinogenic potential. Clearly, the benefits of mammography outweigh the risks, considering the reduced amount of ion radiation to which women are exposed from mammography versus the tremendous improvement in quality-control aspects of screening mammography.

Epidemiologic studies of occupational exposures to radiation have been targeting radiologists since the early twentieth century. These niche groups of physicians have higher incidences of lung, pancreas, thyroid, bone, and breast cancers than practitioners in other medical specialties. Occupational exposure to ionizing radiation is highest among underground uranium miners, commercial nuclear power plant workers, fuel fabricators, physicians, flight crews and attendants, industrial radiographers, and well loggers.[100] Other populations of interest include victims of the atomic bombings in Nagasaki and Hiroshima in World War II. The high doses of ionizing radiation contribute to cancers of the lung, breast, colon, ovary, stomach, and thyroid.

Non-ionizing radiation

Non-ionizing radiation includes microwaves, radio waves, and extremely low doses of electromagnetic fields (EMF). Early epidemiologic studies observed that residential exposure to the weak EMF surrounding power lines was associated with a small elevated risk of childhood cancers. When these studies have been evaluated for their validity and study design in two large case-control studies, no detectable effect of residential magnetic field exposure was found on the development of brain tumors in children.[101,102]

Cell phone usage has increased to include 65% of all U.S. households, up from 62% in 2002. As cellular telephones are a relatively new technology, no long-term follow-up on their biological effects is possible as yet. However, the lack of ionizing radiation and the low-power frequency EMF level emitted from cell phones and absorbed by human tissues make it unlikely that these devices can cause cancer.[103] Moreover, several well-designed epidemiologic studies have failed to find any consistent association between cell phone use and head and neck cancers.[104–106] It is impossible to prove that any product or exposure is absolutely safe, especially in the absence of very long-term follow-up. Nevertheless, the scientific data do not demonstrate that mobile phones are harmful. If individuals are concerned about the radiofrequency (RF) energy from these products, they might choose digital rather than analog telephones, because the former use lower RF levels.[107]

Ultraviolet radiation

Ultraviolet-A (UV-A) radiation from sunlight can suppress cellular immunity, and the suppression of immunity has been postulated as the factor for tumor growth. UV-A is the major cause of nonmelanoma skin cancer, with cumulative exposure and number of lifetime sunburns being predictive of risk. Incidence of melanoma, the most insidious form of skin cancer, is increasing worldwide more rapidly than incidence of any other cancer; mortality rates are also increasing by about 2% per year for this form of skin cancer.

Conversely, sunlight has been shown to protect against cancer development. Epidemiologic studies of prostate, breast, and colon cancer suggest an inverse relationship between sunlight exposure and the incidence and mortality rates for these diseases. Sunlight activation of vitamin D has been shown to retard the growth of colon and breast cancer cells. Unlike the zero tolerance given to tobacco products, it would be remiss to promulgate public health messages to completely avoid sunlight to people who have suffered little or no skin damage from UV-A.[108]

Drugs

Despite the vast array of chemicals discovered to cause cancer in animals, few chemicals (other than tobacco) exist for which there is strong evidence of causation of the common cancers in humans. Medications associated with malignancies include analgesics, cyclophosphamide, and barbiturates, which have been associated with an increased risk (or, in the case of barbiturates, decreased risk) of bladder cancer. Analgesics such as phenacetin have been linked to tumors of the renal pelvis, ureter, and urinary bladder.[109] Cyclophosphamide, an immunosuppressive drug used for the treatment of non-Hodgkin's lymphoma, is prescribed to 500,000 patients annually worldwide. The cumulative risk of bladder cancer in patients taking cyclophosphamide was found to be 10.7% at 12 years of follow-up.[110] Barbiturates, such as phenobarbital, have been shown to interact negatively with smoking in bladder cancer risk. This barbiturate deactivates bladder carcinogens found in tobacco smoke.[111]

Nonsteroidal anti-inflammatory drugs

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most frequently used drugs in the United States. It is has been estimated that 75 million prescriptions for these agents are dispensed annually at a cost of about $2.5 billion.[112] NSAIDs were first introduced in 1949 for their anti-inflammatory properties in the treatment of arthritis. The term "NSAIDs" applies to all "aspirin-like" drugs that are used clinically as antipyretics, analgesics, and anti-inflammatory agents. The drugs inhibit enzymes of the cyclooxygenase (COX) family and, in doing so, prevent the production of certain eicosanoids (a large family of intracellular signaling molecules) in response to inflammatory or mitogenic stimuli.[113]

The antitumor effects of NSAIDs have been extensively studied in the last 25 years. Numerous observational and case-control studies reported since 1989 indicate that regular NSAID use is associated with a reduced risk of colorectal adenomas, cancer, and cancer mortality.[114] Antitumor effects have been associated with NSAID-mediated inhibition of COX activity. In particular, these drugs are linked to up-regulation of COX-2, an enzyme associated with tissue regulation of inflammation.[115] COX-2 is found wherever inflammation is present; it is markedly up-regulated in major epithelial cancers, including colon, esophagus, lung, breast, and prostate cancer. COX-2 may be a key component of epithelial tumorigenesis and its suppression of NSAIDs.

Observational evidence collected as part of epidemiologic studies indicates that regular use of nonspecific COX-inhibiting drugs, such as celecoxib, a commonly used COX-2 inhibitor, discourages polyp and tumor growth.[116] Patients taking aspirin or NSAIDs on a regular basis have roughly 50% less risk of developing colorectal polyps. Additionally, investigators have noted a 50% reduction in the incidence of carcinoma and in cancer-associated mortality with use of such drugs.[117]

Exogenous hormones

Combined oral contraceptives (OC) and postmenopausal hormones are the most vital source of exogenous estrogens for women today. In the past, synthetic hormones such as diethylstilbestrol (DES) were widely prescribed for the prevention of miscarriage and to suppress lactation. Epidemiologic studies of the risk of breast cancer in mothers exposed to DES during their reproductive and pregnancy years suggest a modest 20%–50% increased risk among the exposed women, with a latency period of about 15 to 20 years.

More than 50 epidemiologic studies have evaluated the relationship between OC use and breast cancer risk. Combined OCs contain ethyl estradiol and a progestin. The role of contraceptives in breast cancer risk is controversial and not clearly established, with most studies showing no relationship,[118,119] no significant increase in breast cancer risk with long duration of use, or a small increased risk of breast cancer based on the recency of OC use. The risk associated with OC use

among current users was found to persist for ten years after discontinuation, yet no risk was associated with duration of use, age at first use, or dose and formulation. The study results suggest that the pattern of risk seems incompatible with a genotoxic effect, and that OC use may act as a late-stage promoter of preexisting tumors. Case-control studies have consistently demonstrated that the use of such contraceptives reduces the risk of endometrial cancer by 50% and the risk of ovarian cancer by 40%.

The association between hormone replacement therapy (HRT) and breast cancer is an issue of great public health importance, given the increasing size of the older female population. A large effect of HRT on breast cancer risk has not been detected. Some risk may be associated with current or long-term HRT among women who receive it for five to ten years or longer.[120] One factor to consider in these studies is detection bias. Current users of hormones must see a physician to review prescriptions and, therefore, are more likely to be screened for breast cancer.

Tamoxifen is a nonsteroidal antiestrogen medication that has been used successfully for 15 years in the treatment of breast cancer. Because tamoxifen acts by binding to estrogen receptor sites, it has been most effective in treating postmenopausal women, who are more likely to have cancers containing estrogen receptors. In contrast to its antiestrogenic tumor-suppressor action in the case of breast cancer, this drug has been associated with the development of endometrial carcinoma.[121] The trade-off between the effective use of tamoxifen in breast cancer prevention and the higher risk of endometrial cancer leads to a recommendation for routine screening for the latter cancer in tamoxifen users.

_/ Biomarkers

As many as 80% of cancer cases are theoretically preventable because the controlling causative factors are exogenous rather than inborn or inherent. We can estimate that in the absence of external carcinogenic exposures resulting from lifestyle, occupation, and the ambient environment, 400,000 of the annual 500,000 cancer-related deaths in the United States could be averted. More effective methods are needed to identify groups and individuals at greatest risk of cancer at a stage where intervention is possible.

The field of molecular epidemiology offers a potentially powerful tool in cancer prevention by combining biomarkers, measurement of carcinogenic dose, biologic response, and susceptibility with epidemiologic methods. Biomarkers offer a strategy to

Table 3-6 Examples of Biomarkers of Internal Dose

Biomarker	Source of Exposure	Biologic Sample
Aflatoxin	Contaminated food	Urine
Bacterial mutations	Cigarette smoke	Cervical fluids
Benezene, toluene	Cigarette smoke	Urine, breath concentration
CFA	Occupational exposure	Urine
Cotine	Cigarette smoke	Serum, urine, saliva
DNA sequences	HPV	Cervicovaginal lavage
HDL Alkaline phosphatase	Alcohol	Serum
Lead-210	Cigarette smoke	Bone, soft tissues
Mutagens	Cigarette smoke, various occupational exposures	Urine
Nitrosamino acids	N-nitroso compounds in diet	Urine
Selenium	Diet	Hair, toenails
Vitamin levels	Diet	Serum

CFA: 3-chloro-4-fluoroaniline
HPV: human papillomavirus
HDL: high-density lipoprotein

Nasca PC: Biomarkers and epidemiological studies of cancer, in Nasca PC, Pastides H (eds): *Fundamentals of Cancer Epidemiology*. Sudbury, MA, Jones and Bartlett, 2001, pp 85–102; Perera FP: Molecular epidemiology in cancer prevention, in Schottenfeld D, Fraumeni JF (eds): *Cancer Epidemiology and Prevention*. New York, Oxford University Press, 1996, pp 101–115.

assess precursors of disease and identify biologic markers of exposure. Traditional epidemiologic tools such as questionnaires and medical records are important for measuring the external dose of a particular environmental exposure. Because these epidemiological measures rely on human recall, however, a certain amount of misclassification can be expected to occur.

The term *biomarker* is used to describe the application of chemical, physical, radiologic, and immunobiologic tests to human biologic samples, such as blood, urine, and tissue. Table 3-6 provides examples of biomarkers that measure internal dose. In biomarkers of internal dose, the investigator examines the extent to which the biomarker correlates with the epidemiological measure. The accuracy of the epidemiological exposure data can be assessed by measuring body burden levels of the actual compound or one of its stable metabolites in human tissue.[122] Examples of biomarkers include plasma or salivary cotine from cigarette smoke, urinary aflatoxin indicative of dietary exposure, and N-nitroso compounds in urine from dietary sources and cigarette smoke.[123]

Table 3-7   Probability of Developing Invasive Cancers Over Selected Age Intervals, by Sex, US, 1998-2000*

		Birth to 39 (%)	40 to 59 (%)	60 to 79 (%)	Birth to Death (%)
All Sites†	Male	1.36 (1 in 73)	8.03 (1 in 12)	33.92 (1 in 3)	44.77 (1 in 2)
	Female	1.92 (1 in 52)	9.01 (1 in 11)	22.61 (1 in 4)	38.03 (1 in 3)
Bladder‡	Male	.02 (1 in 4603)	.40 (1 in 250)	2.36 (1 in 42)	3.46 (1 in 29)
	Female	.01 (1 in 9557)	.12 (1 in 831)	.64 (1 in 157)	1.10 (1 in 91)
Breast	Female	.44 (1 in 229)	4.14 (1 in 24)	7.53 (1 in 13)	13.36 (1 in 7)
Colon &	Male	.06 (1 in 1678)	.806 (1 in 116)	3.94 (1 in 25)	5.88 (1 in 17)
rectum	Female	.06 (1 in 1651)	.67 (1 in 150)	3.05 (1 in 33)	5.49 (1 in 18)
Leukemia	Male	.15 (1 in 649)	.20 (1 in 495)	.82 (1 in 122)	1.45 (1 in 70)
	Female	.13 (1 in 789)	.14 (1 in 706)	.46 (1 in 219)	1.00 (1 in 100)
Lung &	Male	.03 (1 in 3439)	1.02 (1 in 98)	5.80 (1 in 17)	7.69 (1 in 13)
bronchus	Female	.03 (1 in 3046)	.79 (1 in 126)	3.93 (1 in 25)	5.73 (1 in 17)
Melanoma	Male	.12 (1 in 809)	.49 (1 in 205)	.97 (1 in 103)	1.81 (1 in 55)
of skin	Female	.19 (1 in 532)	.39 (1 in 255)	.51 (1 in 197)	1.22 (1 in 82)
Non-Hodgkin's	Male	.14 (1 in 739)	.45 (1 in 224)	1.27 (1 in 79)	2.10 (1 in 48)
lymphoma	Female	.08 (1 in 1258)	.30 (1 in 332)	.98 (1 in 102)	1.76 (1 in 57)
Prostate	Male	.01 (1 in 12833)	2.28 (1 in 44)	14.20 (1 in 7)	17.15 (1 in 6)
Uterine cervix	Female	.16 (1 in 632)	.31 (1 in 322)	.27 (1 in 368)	.78 (1 in 128)
Uterine corpus	Female	.05 (1 in 1832)	.69 (1 in 144)	1.57 (1 in 64)	2.60 (1 in 38)

*For those free of cancer at beginning of age interval. Based on cancer cases diagnosed during 1998-2000. The "1 in" statistic and the inverse of the percentage may not be equivalent due to rounding.
†All sites exclude basal and squamous cell skin cancers and in situ carcinomas except urinary bladder. ‡Includes invasive and in situ cancer cases.

Data from DEVCAN: Probability of Developing or Dying of Cancer Software, Version 5.1. Statistical Research and Applications Branch, National Cancer Institute, 2003, http://srab. cancer.gov/devcan, and the American Cancer Society, Surveillance Research, 2004: http://www.cancer.org/downloads/STT/CAFF_final PWSecured.pdf. Accessed August 18, 2004.

Host Characteristics Influencing Cancer Susceptibility

Age

Age is a major risk factor for many health outcomes and is frequently associated with numerous exposures. Even if the effect of age is not among the primary objectives of the study, it is important to assess its relationship with exposures and outcomes, given its potentially confounding effects. The age distribution of new cancer cases by site reveals that leukemia is the leading cancer for individuals younger than age 20. New cervical cancers remain high in women aged 20–54, whereas the highest distribution of new testicular cancer cases occurs in the 20–34 age group.[124] An estimated one-third of deaths in children younger than age 14 involve leukemia.[125] As shown in Table 3-7, the greatest number of cancer deaths predicted for males in 2004 were expected from prostate cancers in the 60–79 age group. Women in the same age strata are predicted to primarily die of cancer of the uterine corpus to cancer of the breast.[124]

Because age is such an important determinant of cancer risk, it is critical in epidemiological studies to make adjustments for age in the statistical analysis, unless comparison groups have the same age distribution.
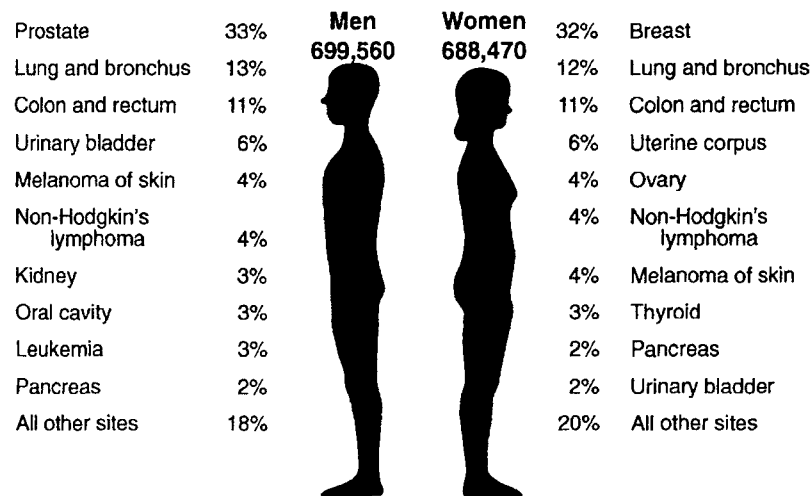
Sex

The distributions of new cancer cases in each sex are shown in Figure 3-5. The prostate is the leading site for cancer in men, followed by lung and bronchus and colorectal cancer. The leading site of new cancer cases in women is the breast, followed by lung and bronchus and colorectal cancer. Figure 3-6 shows the estimated number of cancer deaths by gender. The majority of cancer deaths for both genders derive from lung and bronchus cancers, followed by prostate cancers for men and breast cancers for women.[125]

Genetic Predisposition

Genetic epidemiology in cancer research is used to identify inherent susceptibility factors for primary, secondary, and tertiary prevention of cancer. The cumulative body of evidence indicates that genetic factors contribute to the development of most cancer cases,

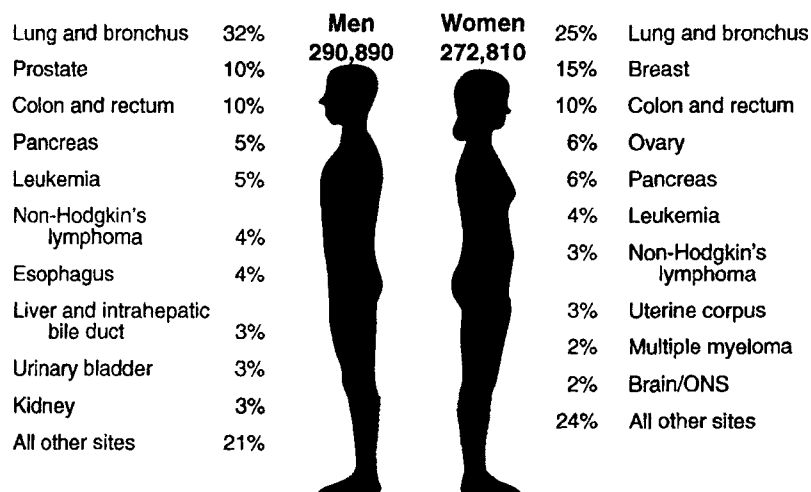| Prostate	33%	**Men** **Women**	32%	Breast
Lung and bronchus	13%	699,560 688,470	12%	Lung and bronchus
Colon and rectum	11%		11%	Colon and rectum
Urinary bladder	6%		6%	Uterine corpus
Melanoma of skin	4%		4%	Ovary
Non-Hodgkin's lymphoma	4%		4%	Non-Hodgkin's lymphoma
Kidney	3%		4%	Melanoma of skin
Oral cavity	3%		3%	Thyroid
Leukemia	3%		2%	Pancreas
Pancreas	2%		2%	Urinary bladder
All other sites	18%		20%	All other sites

Excludes basal and squamous cell skin cancers and in situ carcinomas except urinary bladder.

Figure 3-5   Estimated U.S. cancer cases, 2004. Data from the American Cancer Society: *Cancer Statistics, 2004.* Atlanta, American Cancer Society, 2004.

including those without a clear familial aggregation. Epidemiologic studies of genetics in cancer etiology have been either family studies or genetic biomarker studies. Family studies can provide general information on the role and/or inheritance patterns of genetic factors in the etiology of cancer. Biomarker studies can target specific genetic factors suspected to be responsible for the pathology of cancer. Epidemiological investigation of genetic predisposition to cancer is increasing thanks to developments in molecular biology, which have made it possible to study genetic markers in large populations.[126] The ongoing Human Ge-

nome Project is almost certain to accelerate this work through the discovery of new genes or gene markers associated with increased genetic predisposition for cancer.[127]

Genes have been discovered that are associated with susceptibility to breast cancer and ovarian cancer (*BRCA1* and *BRCA2*), colon cancer (*APC*), and prostate cancer (*HPC1* and *HPC2*). Much work remains to be done to further investigate the effects of these genes, including elucidating how other known risk factors for these cancers modulate the risk conferred by each of these genes.

Lung and bronchus	32%	**Men** **Women**	25%	Lung and bronchus
Prostate	10%	290,890 272,810	15%	Breast
Colon and rectum	10%		10%	Colon and rectum
Pancreas	5%		6%	Ovary
Leukemia	5%		6%	Pancreas
Non-Hodgkin's lymphoma	4%		4%	Leukemia
Esophagus	4%		3%	Non-Hodgkin's lymphoma
Liver and intrahepatic bile duct	3%		3%	Uterine corpus
Urinary bladder	3%		2%	Multiple myeloma
Kidney	3%		2%	Brain/ONS
All other sites	21%		24%	All other sites

ONS = Other nervous system.

Figure 3-6   Estimated U.S. cancer deaths, 2004. Data from the American Cancer Society: *Cancer Statistics, 2004.* Atlanta, American Cancer Society, 2004.

Ethnicity and Race

The U.S. Bureau of the Census classifies race into categories such as white, African American, Asian or Pacific Islander, Mexican American, and Native American. Race is often similar to ethnicity, in that people who come from a particular racial stock may share a common ethnic identification. Caution should be used when trying to classify individuals with mixed racial parentage into a racial group with which they identify. Race does have implications for differences in incidence and prevalence of disease. Racial or ethnic groups may differ in their attitudes toward illness, care seeking, and prevention.

An illustration of the variation of race in cancer incidence and mortality from the Surveillance, Epidemiology, and End Results (SEER) data appears in Figure 3-7. The data on prostate cancer, which can be detected by physical exam and a prostate antigen test (PSA), reveal how cancer mortality adversely affects African Americans. Seventy-three prostate cancer deaths per 100,000 occurred in African American males compared to 30 prostate cancer deaths per 100,000 in white males.[128]

Socioeconomic Factors

Socioeconomic status (SES) is determined by income, education, occupation, or percentage below the poverty level. Lower SES is related to excess mortality, morbidity, and disability rates. Higher-poverty areas are characterized by later-stage diagnosis, poorer survival, and higher mortality rates. A substantial decline in mortality over time occurs in all socioeconomic groups, but a considerable gradient is still evident where the lower-SES group have worse outcomes. In 1999, for all cancers combined in men, the mortality rate was 13% higher in high-poverty areas. All-cancer mortality in women in 1999 was 3% higher in high-poverty areas. Lower-SES groups have a larger proportion of cancers with poorer prognosis in comparison with higher-SES groups. Poorer survival rates in the lower socioeconomic classes may be attributed to delay in seeking health care, health system barriers (e.g., lack of access to care and lack of insurance), and lack of information about cancer detection and treatment. For example, these high-poverty areas have substantially lower rates of mammography and colorectal cancer screening.[129] Residents of high-poverty areas are also less likely to receive optimal surgical treatment for breast, prostate, and lung cancers.
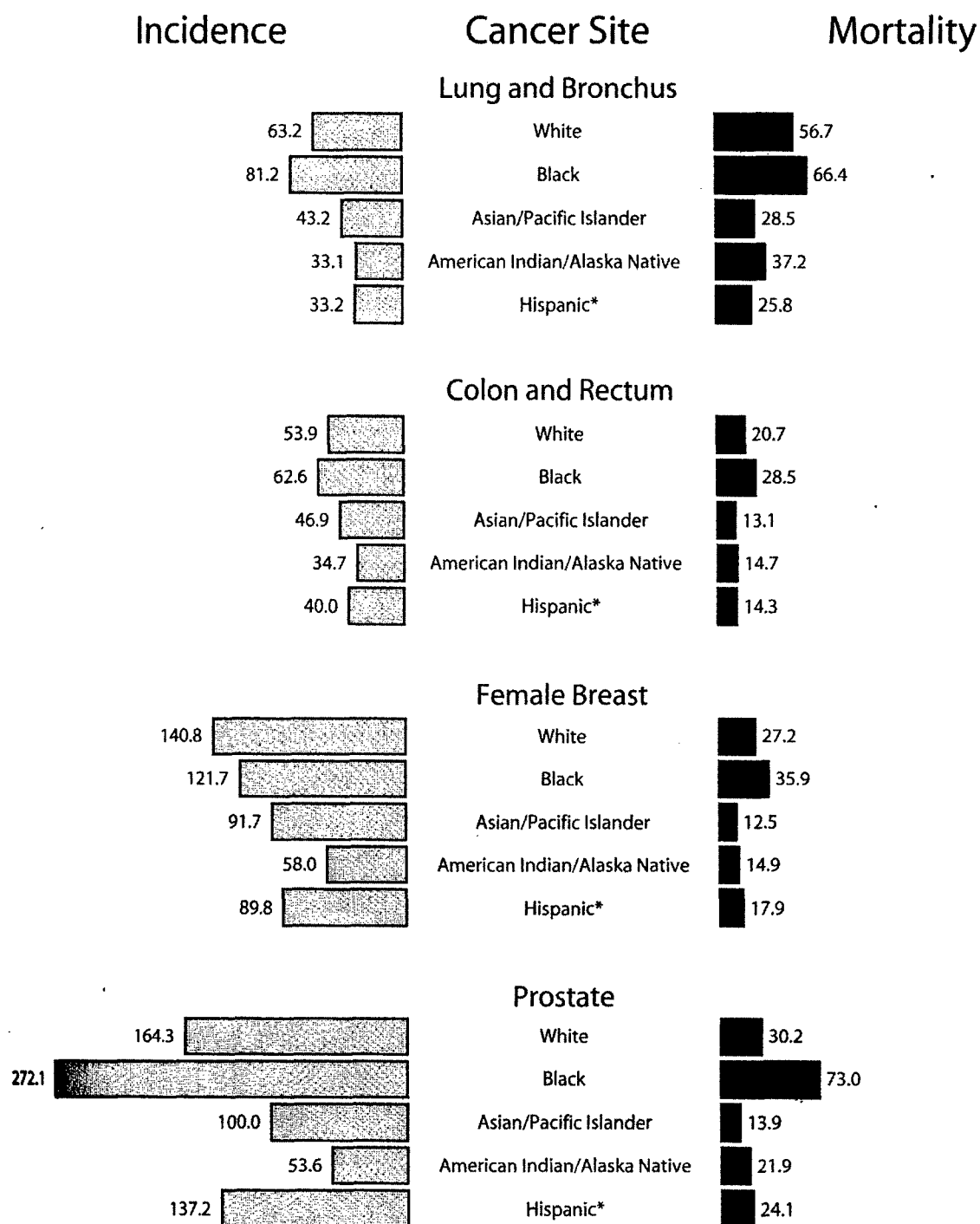
Reproductive History

Factors related to reproduction and sexual behaviors have been identified only for cancers in women. Earlier menarcheal age, later menopausal age, parity status, years of breast-feeding, and later age at first live birth have been associated with breast, endometrial, and ovarian cancers.[130-133]

Cervical cancer has a very different pattern, with multiple sexual partners being identified as a major risk factor. The number of sexual partners is a measure of the likelihood that an individual has been exposed to HPV, which has been implicated as a cause of cervical dysplasia.[8,134,135]

Other Applications of Epidemiology in Oncology

Cancer Pain

One facet of research that is entrenched in cancer nursing practice is determining the epidemiology of cancer pain. The undertreatment of cancer pain is a significant clinical problem. Unrelieved pain has serious negative consequences (e.g., depression, fatigue, and decreases in quality of life for both cancer patients and their family caregivers).[136] Epidemiological research in this area is deemed necessary to define the scope and magnitude of the problem. Epidemiological studies of cancer pain tend to focus on behavioral epidemiology, improving patient and family caregiver knowledge and attitudes, and changing their behaviors regarding cancer pain management. Randomized clinical trials (RCTs) have been undertaken to show that increasing patient or family caregiver knowledge and improving behaviors can significantly affect cancer pain management. RCTs comprise a target intervention in which all study participants in the intervention group receive an identical intervention, contrasted with a tailored intervention in which the study participants receive an intervention structured to meet their specific learning needs. These clinical trials evaluate the effectiveness of an intervention by randomly allocating subjects to groups that will undergo or not undergo a particular intervention.[11] Such studies evaluating the effectiveness of an intervention on attitudes and knowledge regarding the epidemiology of cancer pain were shown to be more successful if they employed a tailored intervention including teaching sessions, home visits, and follow-up phone calls.[137-139]

Incidence   Cancer Site   Mortality

Lung and Bronchus

Incidence	Race	Mortality
63.2	White	56.7
81.2	Black	66.4
43.2	Asian/Pacific Islander	28.5
33.1	American Indian/Alaska Native	37.2
33.2	Hispanic*	25.8

Colon and Rectum

Incidence	Race	Mortality
53.9	White	20.7
62.6	Black	28.5
46.9	Asian/Pacific Islander	13.1
34.7	American Indian/Alaska Native	14.7
40.0	Hispanic*	14.3

Female Breast

Incidence	Race	Mortality
140.8	White	27.2
121.7	Black	35.9
91.7	Asian/Pacific Islander	12.5
58.0	American Indian/Alaska Native	14.9
89.8	Hispanic*	17.9

Prostate

Incidence	Race	Mortality
164.3	White	30.2
272.1	Black	73.0
100.0	Asian/Pacific Islander	13.9
53.6	American Indian/Alaska Native	21.9
137.2	Hispanic*	24.1

*Hispanic is not mutually exclusive from whites, blacks, Asian/Pacific Islanders, and American Indians/Alaska Natives. Incidence data for Hispanics exclude Detroit and Hawaii. Mortality data for Hispanics exclude cases from Connecticut, Oklahoma, New York, and New Hampshire. Rates are per 100,000 and age-adjusted to the 2000 U.S. standard population by five-year age groups.

Figure 3-7   SEER cancer incidence and U.S. death rates, 1996–2000 (by cancer site and race). Data from SEER 12 areas and NCHS public use data file.

Application of Epidemiology to Nursing Practice

Nursing professionals play integral roles in all aspects of cancer prevention and cancer control. Nurses have played major roles in the development of effective means to educate, prevent, and detect cancers early.

Much of the progress that has been made in cancer control has stemmed from epidemiologic research that aims to understand environmental, genetic, and population risks for developing specific cancer(s). Nurses are constantly challenged to construct and interpret cancer risk assessments for patients and their families. This effort demands that nurses be able to accurately interpret epidemiologic studies of cancer risk.

Nurses have implemented the principles of primary, secondary, and tertiary cancer prevention through individualized cancer risk assessment and screening programs, cancer genetics counseling programs, government-sponsored programs, and programs that are offered at public events. A great need exists for nursing professionals to coordinate, implement, and help to provide cancer-control and education services.[140]

Nurses need to consider several common themes as they begin to design programs for cancer control. Some inherent cancer-control themes include consideration of the target population, resources of the institution or sponsor, opportunities for and value of collaboration, and resources for and approaches to public education, funding, marketing, and program evaluation.[141]

As cancer prevention and early detection continue to grow as a priority for oncology professionals and the institutions where they work, nurses will continue to play a critical role in the development, management, and success of cancer-control programs.

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Body weight and body fat distribution in relation to lifestyle factors among Chinese women
in Shanghai
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Historically, the prevalence of obesity has been low among Chinese women. Over the past 20 years, the prevalence of obesity has increased markedly, although the prevalence is still much lower than their western counterparts. The Shanghai Women's Health Study (SWHS) provides a unique opportunity to explore the factors related to this rapid change. The baseline survey data from the SWHS were used to investigate the factors including demographic, lifestyle and reproductive factors that correlate with Chinese women's body weight and fat distribution. The SWHS was initiated in 1997 and completed in 2000 with 75,049 women aged 40-70 recruited (response rate of 93%). Detailed information on demographic, tobacco and alcohol use, physical activity, menstrual and reproductive history was collected from all study participants during an in-person interview using a structured questionnaire. A 77-item quantitative food questionnaire was used to collect information on usual diet and intake. Height, weight, and waist hip circumferences were measured at interview. Overweight status was defined as a body mass index (BMI) of 27-29.99 kg/m^2, obesity status was defined as BMI > 30 kg/m^2 and central obesity was defined as waist to hip ratio of > 0.84. Thirteen percent of women in the SWHS were overweight and 5% were obese. Twenty-eight percent of women were identified as having central obesity. Chi-squares were used to compare differences in frequency distribution of obesity by lifestyle, dietary and reproductive variables. Polychotomus logistic regression was applied to identify factors that significantly contribute to the variation of BMI and WHR. We found that income, education, dietary fat intake and regular exercise were inversely related to the risk of both general obesity and central obesity while high number of live births, long duration of breast feeding, high caloric and carbohydrate, cigarette smoking, non-professional occupation, and daily television were associated with an increased risk of obesity, although the associations are generally weaker for central obesity. Earlier menarche and later menopause were only related to increased risk of general obesity. This study provides important information for obesity prevention among Chinese women.

INTAKE OF FRUITS, VEGETABLES AND SELECTED MICRONUTRIENTS IN RELATION TO THE RISK OF BREAST CANCER

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High fruit and vegetable intake has been linked with a reduced risk of breast cancer, but evidence is not consistent. We investigated the associations of breast cancer risk with vegetables, fruits and related micronutrient intake in a population-based case–control study among Chinese women in Shanghai, where dietary patterns differ substantially from other study populations. Included in the study were 1,459 incident breast cancer cases and 1,556 frequency-matched controls. Usual dietary habits were assessed by in-person interviews. Logistic regression was used to compute adjusted odds ratios (ORs) and 95% confidence intervals (CIs) to measure strength of the associations. There was no association between breast cancer risk and total vegetable intake. The risk of breast cancer declined, however, with increasing intake of dark yellow-orange vegetables (trend test, $p = 0.02$), Chinese white turnips (trend test, $p \leq 0.001$), and certain dark green vegetables (trend test, $p \leq 0.001$) with adjusted OR in the highest quintile being 0.79 (95% CI = 0.60–0.98) ,0.67 (95% CI = 0.53–0.85) and 0.65 (95% CI = 0.51–0.83) respectively. Intake of fruits, except watermelons and apples, was inversely associated with breast cancer risk (p-values for trend tests ≤ 0.05). Our study suggests that high intake of certain vegetables and fruits may be associated with a reduced risk of breast cancer.
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Key words: *fruit; vegetable; micronutrient intake; breast cancer risk*

Vegetables and fruits contain numerous bioactive and potentially anticarcinogenic substances including carotenes, dithiolthiones, flavoids, indoles, isothiocyanates, phenols, folic acid and vitamins C and E.[1] There are many possible mechanisms by which the above substances might inhibit carcinogenesis, such as antioxidant effects, increases in cell-to-cell communication, activation of enzymes involved in carcinogen detoxification, alteration of estrogen metabolism, effects on DNA methylation and repair and antiproliferative effects.[1,2] Epidemiologic studies on the relationships of intake of vegetables and fruit with breast cancer risk, however, have not been consistent. Several studies reported an inverse association with vegetable[3–15] or fruit intake.[3,4,8,10–12, 14, 16,17] Other studies reported a non-significant inverse association of fruit and vegetable intake with breast cancer.[12,23,33] The results from previous studies on micronutrients such as vitamin A, C and E and carotene have also been inconsistent.[3,4,12–14,16,18–26] A recent pooled analysis of 8 cohort studies showed no significant association between fruit and vegetable intake and breast cancer risk.[27] Virtually all the above studies, however, were conducted among Caucasian women. Many studies evaluated only a limited number of food items. One of the primary aims of the study is to examine whether intake of certain fruits and vegetables may be related to a reduction of breast cancer risk. During 1996–98 we conducted a large population-based case–control study among Chinese women in Shanghai, where the incidence rate of breast cancer is about one-third the rate in US Caucasian women[28] and the dietary practices and foods are not represented by previous studies conducted in western society.

MATERIAL AND METHODS

The Shanghai Breast Cancer Study, a population-based case–control study, was designed to recruit women aged 25–64 who were newly diagnosed with primary breast cancer either invasive or *in situ* between August 1996 and March 1998. All study subjects were permanent residents of urban Shanghai. They had no prior history of any cancer and were alive at the time of interview. Through a rapid case-ascertainment system, supplemented by the population-based Shanghai Cancer Registry, 1,602 eligible breast cancer cases were identified during the study period and in-person interviews were completed for 1,459 (91.1%) of the eligible cases. The major reasons for non-participation were refusal (109 cases, 6.8%), death before interview (17 cases, 1.1%), and inability to locate (17 cases, 1.1%). Two senior pathologists confirmed all diagnoses through review of slides.

The Shanghai Resident Registry, which registers all permanent residents in urban Shanghai, was used to randomly select controls from female residents, frequency-matched to cases by age (5-year intervals). The number of controls in each age-specific stratum was determined in advance according to the age distribution of the incident breast cancer cases reported to the Shanghai Cancer Registry from 1990–93. Only women who had the same address held by the registry during the study period were considered eligible for the study. In-person interviews were completed with 1,556 (90.3%) of the 1,724 eligible controls identified. Reasons for non-participation included refusal (166 controls, 9.6%) or death (2 controls, 0.1%). Trained interviewers measured participants for their current weight, circumference of the waist and hip, and sitting and standing heights. Two measures were taken, with a tolerance <1 cm for height, 0.5 cm for circumferences, and 1 kg for weight. A third measure was taken if the difference between two measures was larger than the tolerance stated above. From these data waist: hip ratio and body mass index (BMI, kg/m^2) were calculated.

A structured questionnaire was used to elicit detailed information on demographic factors, menstrual and reproductive history, hormone use, dietary habits, prior disease history, physical activity, tobacco and alcohol use, weight and family history of cancer. Energy intake was adjusted for in the multivariate analysis using the standard method.[57]

Postmenopausal hormone use was defined as ever using sex hormones 1 month. Very few Chinese women (2.8%) report taking sex hormones preventing us from a detailed analysis. We conducted an analysis excluding the small amount of women who reported using hormone replacement therapy and the results did not change substantially. Menopausal status was defined as not menstruating in the past 12 months. There was not a peri-meno-

pausal category. Alcohol consumption consisted of 2 categories regular alcohol consumption and not regularly consuming alcohol. Regular alcohol consumption was defined as at least 1 time per week for more than 6 months continuously. Physical activity information was obtained from reports of lifetime occupational activity, whereas non-occupational physical activities (*i.e.*, exercise and sports, household and transportation activities [walking, cycling]) were assessed for the adolescent period (13–19 years) and in the 10 years before entering the study (adulthood). Usual dietary habits over the past five years were assessed using an in-person interview with a validated quantitative food frequency questionnaire (FFQ). This FFQ was developed based on data from a 24-hr dietary survey of approximately 400 adult Shanghai residents in a recent validation study conducted in 200 women. Nutrient and food intake assessed by the FFQ and the multiple 24-hr recalls correlated very well, with the correlation coefficients being 0.59–0.66 for macronutrients, 0.41–0.59 for micronutrients, and 0.41–0.66 for major food groups. The FFQ listed 76 food items that cover over 85% of foods consumed in Shanghai. Of these 76 food items, 30 are fresh vegetable food items and 8 are fruit items.[30] The interview was conducted shortly after the breast cancer diagnosis for cases and during this same time period for the controls. Each study participant was first asked how frequently she consumed a specific food or a group of foods, (*e.g.*, daily, weekly, monthly, yearly, or never) followed by a question on how many lians (= 50 g) of food eaten per unit of time (day, week, month, or year) in the previous 5-year period, ignoring any recent changes in usual dietary intake within the 5-year period. Total dietary intake of vitamin A (mg), including total carotene(mg) and retinol(mg), vitamin C (mg) and vitamin E (mg) was calculated based on data from the Chinese Food Composition Table.[29]

Statistical analyses were conducted using SAS Version 8.0 (SAS Institute, Cary, NC). Quintile distributions among controls were used to categorize the dietary-intake variables. Odds ratios were used to measure the association of breast cancer risk with intake of selected food groups and vitamins. Unconditional logistic regression was used to model the association between intake of selected vegetables and fruits, micronutrients and breast cancer risk. Maximum likelihood estimates of the odds ratios (ORs) and 95% confidence intervals (CIs) were calculated adjusting for those potential confounding variables that were statistically significant as shown in Table I. Age was included as a continuous variable throughout data analyses. Trend tests were conducted by treating categorical variables as the ordinal values of the quintile levels in the models. Analyses were also stratified by menopausal status. All statistical tests were based on two-sided probability and a significance level of ($p \leq 0.05$).

RESULTS

Table I shows comparisons of cases and controls on selected demographic factors, known risk factors of breast cancer, total energy and fat intake. Compared to controls, cases had earlier age at menarche, later age at menopause and later age at first live birth. Cases were more likely to have a higher education, a family history of breast cancer among first-degree relatives, a history of breast fibroadenoma, a higher body mass index (BMI), a higher waist-hip-ratio (WHR), and were less likely to exercise in the past 10 years. All of the above variables were considered potential confounders and adjusted for in subsequent analyses. No significant differences between cases and controls were observed for family income, adult height, usual intake of energy and fat, or percentage of calories as fat. Cases median intake of dark green vegetables without bok choy was 7.2 g/day; dark yellow vegetables 2.4 g/day; citrus fruit, 9.0 g/day; grapes, 2.3 g/day; banana, 2.4 g/day; peach, 2.3 g/day and watermelon was 105.0 g/day (Table II).

Overall, there was no association between total vegetable intake and breast cancer risk (Table III). Increased consumption of dark green vegetables (other than bok choy), dark yellow-orange vegetables and white turnips, however, was inversely associated with

TABLE I. – COMPARISON OF CASES AND CONTROLS ON DEMOGRAPHICS AND SELECTED BREAST CANCER RISK FACTORS[1]

	Cases[2] (n = 1,459)	Controls[2] (n = 1,556)	*p*-value
Age	47.8 ± 8.0	47.2 ± 8.8	0.03
Education (%)			
No formal education	3.6	5.5	0.01
Elementary school	8.5	8.4	
Middle + high school	74.3	75.4	
Profession, college and above	13.6	10.7	
Per capita income (Yuan) (%)			0.05
<4000	19.8	18.2	
4000–5999	31.7	31.9	
6000–7999	13.0	13.9	
8000–8999	20.2	23.5	
≥9000	15.2	12.4	
Breast cancer in first degree relatives (%)	3.7	2.4	0.05
Ever had breast fibroadenoma (%)	9.6	5.0	<0.01
Exercised regularly (%)	18.8	25.2	<0.01
Weight	61.8 ± 47.2	58.7 ± 25.4	0.03
Height	160.5 ± 35.2	159.1 ± 22.0	0.20
Regular smoker (%)	2.6	2.5	0.86
Hormone use (%)			
OC	21.9	20.9	0.51
HRT	2.9	2.7	0.76
Regular drinker (%)	4.0	4.1	0.44
Body mass index	23.5 ± 3.4	23.1 ± 3.4	<0.01
Waist-to-hip ratio	0.81 ± 0.06	0.80 ± 0.06	<0.01
Nulliparous (%)	5.1	3.9	0.13
Age at first live birth[3] (years)	26.8 ± 4.2	26.2 ± 3.9	<0.01
Menarcheal age (years)	14.5 ± 1.6	14.7 ± 1.7	<0.01
Menopausal age[4] (years)	48.1 ± 4.6	47.5 ± 4.9	0.02
Energy intake (kcal/day)	1,871 ± 464.5	1,845.1 ± 463.6	0.13
Total fat intake (g/day)	36.3 ± 17.4	35.3 ± 16.2	0.08
Total fat calories (%)	17.1%	16.9%	0.43

[1]The Shanghai Breast Cancer Study, 1996–1998. Subjects with missing values were excluded from the analysis.–[2]Unless otherwise specified, values are mean ± SD.–[3]Among women who had live births.–[4]Among post-menopausal women.

TABLE II – INTAKE OF FRUIT/VEGETABLES AMONG CONTROLS[1]

Items	Mean ± SD (g/day)	Median (25th, 75th) (g/day)
Vegetables (total)	269.6 ± 167.1	234.5 (158.8, 333.8)
Dark green[2]	88.41 ± 70.3	72.7 (38.2, 123.8)
Bok choy	75.0 ± 62.0	57.8 (28.9, 115.7)
Others	13.4 ± 30.7	8.1 (4.0, 16.0)
Dark yellow[3]	6.6 ± 14.7	2.5 (0.6, 6.3)
Cruciferous[4]	98.8 ± 71.7	84.4 (46.1, 132.7)
Fresh legumes[5]	21.2 ± 29.5	81.5 (50.8, 127.4)
Allium[6]	8.8 ± 9.5	6.1 (3.9, 10.4)
Mushrooms	7.6 ± 12.8	4.0 (1.9, 8.9)
White turnip	4.3 ± 9.0	1.9 (0.5, 3.9)
Tomatoes	30.8 ± 43.7	17.3 (6.7, 34.6)
Melons[7]	43.3 ± 46.2	30.4 (15.7, 54.1)
Others[8]	37.7 ± 33.3	28.1 (16.4, 47.5)
Fruits (total)	223.3 ± 170.5	190.5 (114.7, 289.0)
Citrus fruits[9]	20.2 ± 25.7	12.7 (3.6, 27.1)
Apples	32.0 ± 39.8	19.0 (5.3, 47.5)
Watermelon	119.2 ± 103.4	93.3 (46.6, 163.3)
Grapes	8.9 ± 20.0	2.6 (0.6, 9.5)
Bananas	8.9 ± 17.6	3.2 (0.8, 8.4)
Peach	6.8 ± 13.8	2.3 (0, 6.1)
Pear	16.8 ± 23.8	8.6 (2.2, 20.0)
Others[10]	10.3 ± 19.3	4.7 (1.2, 10.2)

[1]The Shanghai Breast Cancer Study, 1996–1998.–[2]Dark green vegetables: bok choy, spinach, fresh green pepper, garlic shoots, chives, scallions, Chinese celery.–[3]Dark yellow: carrots, sweet potato.–[4]Cruciferous vegetables: bok choy, cabbage, napa cabbage, cauliflower, Chinese white turnip.–[5]Fresh legumes: fresh soybean, fresh broad beans, yard long bean, green bean, hyacinth bean/snow peas.–[6]Allium vegetables: garlic, head of garlic, chives, scallions, garlic shoots.–[7]Melons: winter melon, cucumber, wax gourd.–[8]Other vegetables: potato, eggplant, corn, ginger, lotus root, wild rice stems, asparagus lettuce, bamboo shoots.–[9]Citrus Fruits: tangerines, oranges, grapefruits.–[10]Other fruits: strawberries, cantaloupe.

breast cancer risk. The ORs and 95% CI for the highest quintile intake of these vegetables were 0.65 (0.51–0.83), 0.79 (0.60–0.98) and 0.67 (0.53–0.85), respectively. Total fruit consumption was not associated with breast cancer risk. Intakes of all individual fruits, except watermelon and apple, had an inverse association with breast cancer risk. Total fruit without watermelon displayed a strong significant inverse association (OR = 0.77, 95% CI = 0.60–0.98) (trend test p = 0.02). Further analyses were conducted stratified by menopausal status (data not shown on tables). Both dark yellow-orange vegetables and citrus fruits were found to be inversely associated with breast cancer risk among pre-menopausal women, with adjusted ORs in the highest quintile being 0.66 (95% CI = 0.49–0.96) for dark yellow-orange vegetables (trend test, p = 0.003) and 0.65 (95% CI = 0.48–0.88) for citrus fruits (trend test, p = <0.001). These inverse associations, however, were not statistically significant in post-menopausal women, 1.04 (95% CI = 0.70–1.55) for dark yellow-orange vegetables (trend test, p = 0.88) and 0.78 (95% CI = 0.53–1.16) for citrus fruits (trend test, p = 0.23) in the highest quintiles, respectively.

Further adjustment for family income, adult height, usual intake of energy and fat or percentage of calories of fat did not appreciably change the risk estimates. For example, ORs from lowest to highest quintile for dark green vegetables with bok choy were 0.83 (0.66–1.04), 0.75 (0.60–0.94), 0.68 (0.54–0.87), 0.65 (0.51–0.83); dark-yellow orange vegetables 0.88 (0.70–1.11), 0.90 (0.71–1.13), 0.77 (0.61–0.98), 0.75 (0.59–0.95); white turnip 0.69 (0.56–0.86), 0.66 (0.52–0.83), 0.58 (0.46–0.74), 0.67 (0.53–0.85); citrus fruits 0.83 (0.66–1.04),0.75 (0.60–0.94), 0.67 (0.53–0.85), 0.68 (0.54–0.85) and watermelon 0.83 (0.66–1.04), 0.93 (0.76–1.14), 0.94 (0.67–1.31), 1.17 (0.94–1.47), respectively. Those risk estimates were very close to those presented in Table III, indicating that the confounding effects of the above-mentioned variables were minimal.

Odds ratios associated with intake of selected micronutrients are shown in Table IV. A significant inverse association between vitamin E intake and breast cancer risk was found (trend test p = 0.01). There was no evidence of an association on breast cancer risk with intake of total carotene, vitamin C, retinol and total vitamin A.

DISCUSSION

This large population-based case–control study was conducted among Chinese women in Shanghai, a population with an abundant fruit and vegetable intake but of low vitamin supplement usage.[2,31] Among controls in Shanghai, the median intake of total vegetables consumed was 235 g/day, with the major contributing food item being cruciferous vegetables (37%) (Table II). Total median fruit intake was 191 g/day with watermelon comprising 53% of fruit intake by weight. In comparison, the dietary patterns in Western society ranged from a median vegetable intake of 77–262 g/day and a fruit intake of 164–355 g/day.[27] The results of our study suggest no overall association of breast cancer risk with total fruit or vegetable intake. A significant inverse relationship was observed between breast cancer risk and intake of certain dark green vegetables, dark yellow-orange vegetables, and Chinese white turnips and all individual fruits except for watermelon and apples. Of the micronutrients examined, dietary vitamin E was related to a reduced risk of breast cancer.

High intake of fruits and vegetables have been consistently shown to be associated with a reduced risk of several cancers, including cancers of the lung, oral cavity, pancreas, larynx, esophagus, bladder and stomach.[1,32] Results from previous epidemiological studies for breast cancer, however, have been inconsistent. At least 11 case–control studies have reported an inverse association of breast cancer with higher intake of certain fruits and vegetables.[4–6,7–11,13,14,17] Other studies reported a non-significant inverse association.[12,23,33] A recent pooled analysis of cohort studies found that neither fruit nor vegetable intake was associated with breast cancer risk.[27] These cohort studies, however, were conducted in Western society where the intake level of fruits and vegetables is relatively homogenous and consumption indigenous Chinese vegetables such as white turnip are low. Further, the quality of dietary data from these cohort studies also differ, as some studies included fewer than 12 food items in the dietary assessment. The food items included in the food frequency questionnaire used in our study, raw fruits and vegetables, and 90% of the dishes prepared in Shanghai are cooked using these food items, this may improve the accuracy in the dietary assessment in our study. Only the edible portion of the fruits and vegetables were considered in calculating the nutrients using the Chinese food composition table.[29] The 2 predominant cooking methods in Shanghai are deep frying and stir-frying, with 94% of the foods being prepared with soybean oil and the remainder prepared with rapeseed, peanut and lard oils.[30]

Vitamin supplements are widely used in many Western societies, and some foods are fortified with various vitamins, such as folic acids. These all affect observational epidemiologic studies to evaluate dietary factors in relation to cancer risk. Most Chinese women have diets composed mainly of unprocessed and unfortified foods; this allows better assessment of nutrient intake and minimizes potential misclassification.[2] In our study population, only 12.8% of cases and 16.1% of controls ever took vitamin supplements. The vitamin supplements ingested by the women in the Shanghai Breast Cancer study were vitamins A, C, E, B and a multivitamin. Women who used supplements were excluded from the micronutrient analysis. This exclusion did not show any difference than the analysis that included supplement users.

Consumption of both dark green (other than bok choy) and yellow-orange vegetables in our study were inversely related with breast cancer risk. These findings are consistent with several other epidemiologic studies investigating the relationship between di-

TABLE III – ADJUSTED ODDS RATIOS AND 95% CLS FOR THE ASSOCIATION OF BREAST CANCER RISK WITH THE INTAKE LEVEL OF SELECTED FRUITS AND VEGETABLE GROUPS[1]

	Q1 (low)	Q2	Q3	Q4	Q5[2]	*p*-value
Total vegetable	1.00	0.98 (0.78–1.25)	1.11 (0.88–1.40)	0.97 (0.76–1.23)	1.05 (0.81–1.40)	0.81
Dark green	1.00	1.04 (0.83–1.30)	0.89 (0.71–1.12)	0.97 (0.78–1.22)	1.02 (0.82–1.28)	0.83
Bok choy	1.00	1.25 (1.00–1.57)	1.07 (0.86–1.34)	1.22 (0.97–1.53)	1.23 (0.95–1.60)	0.16
Other	1.00	0.83 (0.66–1.04)	0.75 (0.60–0.94)	0.69 (0.54–0.87)	0.65 (0.51–0.83)	<0.001
Dark Yellow	1.00	0.89 (0.70–1.11)	0.90 (0.71–1.13)	0.77 (0.61–0.97)	0.79 (0.60–0.98)	0.02
Cruciferous	1.00	1.05 (0.82–1.30)	1.03 (0.81–1.30)	1.20 (0.95–1.52)	1.10 (0.87–1.40)	0.21
Legumes	1.00	1.01 (0.80–1.27)	0.87 (0.68–1.10)	0.95 (0.75–1.19)	0.89 (0.70–1.14)	0.29
Allium	1.00	0.94 (0.75–1.18)	0.83 (0.66–1.03)	0.75 (0.60–0.94)	0.91 (0.73–1.14)	0.10
Others						
Mushrooms	1.00	1.07 (0.85–1.34)	0.89 (0.70–1.12)	0.92 (0.73–1.16)	0.96 (0.76–1.22)	0.41
White Turnip	1.00	0.69 (0.56–0.86)	0.66 (0.52–0.83)	0.58 (0.46–0.74)	0.67 (0.53–0.85)	<0.001
Tomato	1.00	1.06 (0.84–1.33)	0.92 (0.73–1.16)	1.02 (0.80–1.29)	1.19 (0.94–1.51)	0.24
Melons	1.00	0.88 (0.69–1.12)	1.11 (0.88–1.40)	1.12 (0.88–1.41)	1.12 (0.89–1.43)	0.09
Total fruits	1.00	0.86 (0.68–1.08)	0.83 (0.66–1.05)	0.87 (0.69–1.11)	1.01 (0.80–1.28)	0.89
Citrus Fruits	1.00	0.83 (0.66–1.04)	0.75 (0.60–0.94)	0.67 (0.53–0.85)	0.68 (0.54–0.86)	0.002
Apples	1.00	1.11 (0.89–1.38)	0.96 (0.75–1.22)	0.93 (0.74–1.17)	0.86 (0.66–1.11)	0.09
Grapes	1.00	1.03 (0.82–1.30)	0.88 (0.70–1.10)	0.81 (0.64–1.03)	0.86 (0.68–1.10)	0.05
Banana	1.00	0.89 (0.71–1.12)	0.77 (0.62–0.96)	0.62 (0.49–0.80)	0.73 (0.58–0.93)	<0.001
Watermelon	1.00	0.83 (0.66–1.04)	0.93 (0.76–1.14)	0.94 (0.67–1.31)	1.17 (0.94–1.47)	0.08
Peach	1.00	0.89 (0.70–1.12)	0.76 (0.61–0.95)	0.67 (0.53–0.84)	0.83 (0.67–1.04)	<0.001
Pear	1.00	0.99 (0.79–1.25)	1.06 (0.85–1.33)	0.79 (0.63–0.99)	0.86 (0.67–1.09)	0.05
Other	1.00	0.88 (0.70–1.11)	0.89 (0.71–1.11)	0.80 (0.63–1.02)	0.76 (0.60–0.97)	0.02
Total fruits without watermelon	1.00	0.92 (0.73–1.15)	0.78 (0.62–0.99)	0.80 (0.64–1.01)	0.77 (0.60–0.98)	0.02

[1]Shanghai Breast Cancer Study, 1996–1998. 1,459 cases and 1,556 controls. Odds ratios were adjusted for age, education, family history of breast cancer, history of breast fibroadenoma, waist-to-hip ratio, menarche age, physical activity, ever had live birth, age at first live birth, and total energy compared to the lowest quintile group.– [2]Cut-off values for the quintiles, Total vegetable: 143.6, 201.8, 269.2, 369.6; Dark green: 32.9, 60.9, 89.6, 130.4; bok choy: 23.1, 48.2, 121.5, 121.5, other: 3.3, 6.3, 10.6, 18.1; dark yellow: 26, 1.6, 3.7, 7.5; cruciferous: 38.7, 67.6, 99.7, 144.5; legume: 44.5, 67.7, 94.3, 143.7; allium: 3.6, 5.1, 7.3, 11.7; mushroom: 1.6, 3.2, 5.7, 10.3; turnip: 0, 1.3, 2.6, 5.3; tomato: 5.2, 11.5, 23.1, 44.4; melon: 14.1, 24.0, 38.3, 61.4; other veg: 14.6, 22.6, 35.0, 54.5; fruit: 94.7, 161.1, 225.9, 316.8; citrus: 2.7, 7.2, 16.9, 32.6; apple: 3.2, 13.6, 27.1, 57.0; watermelon: 35, 70, 140, 186.7; grape: .23, 2.0, 4.8, 10.2; banana: .65, 2.0, 4.9, 10.5; peach: 0, 1.2, 3.5, 8.4: pear: 1.7, 5.5, 13.3, 28.6; other fruit: 71, 2.5, 5.9, 14.3.

TABLE IV – DIETARY INTAKE OF SELECTED VITAMINS AND CAROTENE AND THE RISK OF BREAST CANCER[1]

	Q1 (Low)	Q2	Q3	Q4	Q5[2]	*p*-value
All subjects (1,459 cases, 1,556 controls)						
Vitamin A	1.00	1.09 (0.85–1.41)	0.95 (0.73–1.23)	1.02 (0.78–1.33)	1.00 (0.75–1.32)	0.78
Retinol	1.00	1.23 (0.95–1.58)	1.00 (0.77–1.28)	1.04 (0.80–1.36)	1.08 (0.82–1.42)	0.92
Carotene	1.00	1.03 (0.80–1.32)	1.08 (0.84–1.39)	0.86 (0.66–1.12)	1.10 (0.85–1.43)	0.93
Vitamin C	1.00	0.84 (0.66–1.08)	0.85 (0.66–1.09)	0.89 (0.69–1.15)	0.88 (0.67–1.15)	0.49
Vitamin E	1.00	0.96 (0.74–1.24)	1.19 (0.92–1.54)	0.72 (0.54–0.96)	0.69 (0.50–0.96)	0.01
Premenopausal women[3] (852 cases, 859 controls)						
Vitamin A	1.00	1.12 (0.81–1.54)	0.91 (0.65–1.28)	0.92 (0.66–1.28)	0.86 (0.61–1.21)	0.18
Retinol	1.00	0.90 (0.77–1.45)	0.90 (0.65–1.24)	0.94 (0.66–1.31)	0.92 (0.65–1.29)	0.43
Carotene	1.00	1.06 (0.78–1.45)	0.98 (0.72–1.35)	0.87 (0.63–1.20)	1.07 (0.77–1.48)	0.83
Vitamin C	1.00	0.83 (0.60–1.13)	0.74 (0.54–1.01)	0.90 (0.65–1.24)	0.87 (0.62–1.21)	0.60
Vitamin E	1.00	0.92 (0.67–1.26)	1.03 (0.75–1.41)	0.67 (0.47–0.95)	0.77 (0.52–1.16)	0.07
Postmenopausal women[3] (421 cases, 447 controls)						
Vitamin A	1.00	1.07 (0.70–1.62)	0.99 (0.64–1.53)	1.23 (0.78–1.84)	1.47 (0.89–2.43)	0.14
Retinol	1.00	1.57 (1.03–2.40)	1.12 (0.73–1.74)	1.19 (0.76–1.86)	1.52 (0.94–2.45)	0.30
Carotene	1.00	0.94 (2.61–1.46)	1.32 (0.84–2.08)	0.80 (0.51–1.25)	1.22 (0.79–1.89)	0.63
Vitamin C	1.00	0.84 (0.55–1.30)	1.05 (0.68–1.64)	0.79 (0.51–1.22)	0.95 (0.61–1.50)	0.68
Vitamin E	1.00	1.03 (0.66–1.63)	1.68 (1.07–2.64)	0.85 (0.52–1.38)	0.65 (0.38–1.11)	0.13

[1]Shanghai Breast Cancer Study, 1996–1998. Adjusted ORs (95% CI) by quintile, total dietary intake. Odds ratios were compared to the lowest quintile and adjusted for age, education, family history of breast cancer, history of breast fibroadenoma, waist-to-hip ratio, menarche age, physical activity, ever had live birth, age at first live birth, menopausal status and total energy.– [2]Cut-off values for the quintiles: vitamin C: 47.6, 65.2, 82.4, 111.4; vitamin A: 80.3, 123.9, 173.5, 286.3; vitamin E: 9.4, 12.1, 15.6, 19.9; retinol: 378.6, 516.1, 1,659.0 867.2; carotene: 1493.2, 2128.0, 2845.0, 3734.6.– [3]Additional adjustment for menopausal status in the analysis with all subjects.

etary intake of dark green vegetables and risk of breast cancer.[4,7,9,10,12–14,34,35] Dark green/yellow-orange vegetables contain high levels of α- and β-carotenes. These carotenoids may protect against cancer via their ability to block damage by free radicals. β-Carotene can be metabolized into vitamin A that plays a role in differentiation of normal epithelial cells. In addition to β-carotene, dark green-leafy vegetables also have a high level of folate and lutein, the latter being an antioxidant carotenoid that has been shown to have cancer inhibitory effects in *in vitro* and animal experiments.[1] High intake of folate from food sources has been shown to be inversely associated with breast cancer risk in several epidemiologic studies,[12,14,20] including our study conducted in Shanghai.[2] We did not find an inverse association of carotene and breast cancer risk in our study. It is possible that other phytochemicals high in dark green vegetables may explain the inverse association.

Although bok choy accounts for over 25% of the overall vegetable intake for Chinese dark green vegetables, it was not associated with breast cancer risk. There is no immediate explanation

for this finding. Other studies have found a positive relationship between esophageal cancers with bok choy[36,37] and an inverse association with prostate cancer.[38] Potential misclassification error for assessing intake level of this vegetable may be high, as this food is consumed in large quantities and high frequency.[2]

In contrast to findings from several other studies,[13,33,39] we did not find an inverse association of intakes of cruciferous vegetables or legumes with breast cancer risk. Chinese white turnip intake, however, was inversely associated with breast cancer risk. Similar findings were reported from previous studies on oral and nasal cancers.[40,41] Although there is no existing research on the association between breast cancer and white turnips, some phytochemicals in white turnips may be protective. Of them, kaempferol, a flavonoid, has been shown to have cancer inhibitory effects.[42,43]

With the exception of watermelon and apple, inverse associations of breast cancer risk were found for intakes of virtually all fruits evaluated in our study, with the strongest association being citrus fruits. Citrus fruits are known for their high content of vitamin C that may protect cell membranes and DNA from oxidative damage.[44] Recently, a wide variety of flavonoids have been identified in citrus fruits, which may also act as antioxidants.[11,12] High intake of phytochemicals from citrus fruits may contribute to the inverse association between breast cancer risk and this group of fruits in our study.

Watermelon, consumed in large quantity in Shanghai, was not found to be related to breast cancer risk in the study. The reason for a lack of association with this fruit is unknown, but measurement error in assessing intake level of watermelon may have contributed to the null association. Watermelon is consumed on a seasonal basis. Intake level is very high despite the exposure time being limited to 2 months. Other studies on watermelon and cancer are limited to food chemistry experiments on the antioxidant capacity of lycopene and β-cryptoxanthin in watermelon to scavenge free radicals.[45-47]

Our study suggested that breast cancer risk may be inversely related to the intake of vitamin E. Vitamin E is a fat soluble vitamin best characterized as a lipid-soluble antioxidant that protects against lipid peroxidation of cell membranes. It also inhibits formulation of carcinogenic nitrosamines and nitrosamides.[48] Pre-

vious observational studies have shown either inverse[13,22,24-26] or no association[3,15,16,18,33,49-54] of vitamin E with breast cancer risk. Freudenheim et al.[12] found a reduction in risk of breast cancer associated with food sources of vitamin E but not with supplements. A case-control study conducted in Uruguay by Ronco et al.[14] showed a significant inverse association with breast cancer risk and vitamin E. Odds ratios for the highest vs. lowest quartile were 0.40 (95% CI = 0.30-0.60) (trend test, $p \leq 0.001$).

The primary concerns of our study are potential selection and recall bias. Because this is a population-based case-control study with high response rates (91.1% and 90.3%, for cases and controls respectively), the threat of selection bias is limited. As in virtually all case-control studies, our study relies on self-reporting of past dietary habits. Patients with cancer may recall diet in a different way than controls, or change their dietary practice after cancer diagnosis.[55] To reduce differential misclassification of dietary intake, we tried to interview patients as soon as possible. As a result, approximately 80% of the cases from Shanghai were interviewed within 4 months of their cancer diagnosis. Non-differential misclassification is another potential concern; it usually attenuates the true association, however, suggesting that some of the null associations observed in our study may be due to this type of error in dietary assessment.

Because all nutrient intakes were calculated from a food-frequency questionnaire, caution should be exercised in the interpretation of the quantities presented. Furthermore, observational studies cannot disentangle whether some known food constituents (e.g., vitamins) themselves are protective or whether they are serving as markers of unidentified components in vegetable and fruit.[44,56]

In summary, our population-based case-control study conducted on Chinese women from Shanghai with a traditionally low risk of breast cancer revealed that dietary intake of certain vegetables and fruits and vitamin E were inversely associated with the risk of breast cancer. Our findings indicate the necessity for future studies on special populations such as Chinese women in Shanghai with lifestyle and dietary habits divergent from Western society to elucidate the etiology of breast cancer.

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Appendix 4: Malin et al, Abstract and corresponding poster presentation " Insulin Resistance, Insulin-Like Growth Factors and Breast Cancer Risk" for DOD Era of Hope Conference, Orlando, Florida 2002.

Insulin Resistance, Insulin-Like Growth Factors and Breast Cancer Risk

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ABSTRACT

Despite evidence from many *in vitro* experiments, limited population-based research has been performed to investigate how insulin interacts with IGFs in the pathogenesis of breast cancer risk. The purpose of this study is to investigate the interaction of insulin resistance and its joint association with insulin-like growth factors (IGFI, IGF2 and IGFBP3) on breast cancer risk. Included in this study were a subset of subjects (397 case-control pairs) from the Shanghai Breast Cancer Study (R01CA64277). Pre-treatment biospecimens and interview data were collected from all breast cancer cases, along with their individually matched controls. Paired t-tests and Wilcoxon rank sum tests were used to compare the differences between cases and controls. Conditional logistic regression was used to compute adjusted odds ratios (ORs) and 95% confidence intervals (CIs) as a measure of the strength of the association. The mean and median levels of C-peptide, IGFI, IGF2, and IGFBP3 were higher in cases than in controls, with the differences being statistically significant. The risk of breast cancer was associated with higher levels of C-peptide and IGFs in the blood, with significant dose-response relationships. There is a statistically significant two to threefold risk of breast cancer for the women in this study, in the highest quartile of C-peptide, IGF1, IGF2, or IGFBP3 compared to women in the lowest quartile. Analyses to examine the joint effects of C-peptide and IGF1, IGF2 and IGFBP3, revealed a strong positive association. The adjusted ORs, were 3.79 (2.03-7.08) for women with both high C-peptide and IGF1; 3.51 (1.69-7.29), the women with both high C-peptide and IGF2; and 4.03 (2.06-7.88) for women with both high C-peptide and IGFBP3. Findings from this study suggest that women with high levels of both C-peptide and IGFs have a substantially higher risk of breast cancer than those with a high level of either C-peptide or IGFs.

BACKGROUND

1) IGF1, IGF2 have been shown in the potent mitogens for a number of breast cancer epithelial cell lines and are detectable in the majority of human breast tumors.

2) Several epidemiologic studies have suggested a positive association between IGFs and breast cancer risk.

3) The link between insulin resistance, IGF1 and risk of breast cancer may relate to the effect of insulin in amplifying the IGF1 action at the breast tissue level. Insulin sensitivity can be measured using blood C-peptide, a marker of insulin secretion.

4) No study has evaluated the potential joint effect of C-peptide and IGFs on breast cancer risk.

METHODS
Study Design

- The Shanghai Breast Cancer Study, a population-based case-control study was conducted during 1996-1998. The age range of participants was 25-64.
- Subjects for this ancillary study: 400 cases and 400 controls.
- Cases – newly diagnosed with a primary breast cancer.
- Controls: matched ±3 years, menopausal status, and date of sample collection ±30 days.

COLLECTION OF DATA AND BIOSPECIMENS

- In-person interview with the following covariates: reproductive history, last 5 year dietary intake, height and weight, physical activity, hormone use, prior medical history, lifestyle factors, family history of cancer.
- Anthropometrics: weight, height, waist to hip circumference (two measures taken).
- Biologic sample collection: fasting blood samples with a short interview to elicit intake of specific foods and use of medication over 7 days that might influence level of biomarkers.
- Biospecimens for cases collected before cancer therapy.

STATISTICAL ANALYSIS

- Log-transformed data was used in the paired student t-tests and Wilcoxon tests to compare the mean and median differences between cases and controls.
- Conditional logistical regression analysis was used to estimate the odds ratios.

RESULTS

Table 1: Comparison of cases and controls on demographics and selected breast cancer risk factors, the Shanghai Breast Cancer Study, 1996-1998.

Table 2: Case-Control Comparison of Means and Medians for blood levels of C-peptide, IGF1, IGF2 and IGFBP: The Shanghai Breast Cancer Study, 1996-1998.

Table 3: Adjusted Odds Ratios (ORs) and 95% Confidence Intervals (CIs) for the association of breast cancer with blood levels of C-peptide, IGF1, IGF2 and IGFBP3: The Shanghai Breast Cancer Study, 1996-1998.

Table 4: Joint Associations of blood levels of C-peptide and IGF1, IGF2, IGFBP3 with Breast Cancer Risk: The Shanghai Breast Cancer Study, 1996-1998.

CONCLUSIONS

- There is a statistically significant, twofold increased risk of breast cancer for the women in this study, in the highest quartiles of C-peptide, IGF1, IGF2 and IGFBP3 compared to women in the lowest quartiles.
- Women with high levels of both C-peptide and IGFs have a substantially higher risk of breast cancer than those with a high level of either C-peptide or IGFs.

Appendix 5: Malin et al, evalution of the synergistic effect of insulin resistance and insulin-like growth factors on the risk of breast carcinoma. *Cancer*, **100** (4): 694-700 (2004).

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Evaluation of the Synergistic Effect of Insulin Resistance and Insulin-Like Growth Factors on the Risk of Breast Carcinoma

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BACKGROUND. The purpose of the current study was to investigate the association between insulin resistance (which was measured using fasting blood C-peptide) and its joint association with insulin-like growth factors (IGF-1, IGF-2, and IGF binding protein-3 [IGFBP-3]) on the risk of breast carcinoma.

METHODS. Included in the current study were 400 case–control pairs from the Shanghai Breast Cancer Study. Pretreatment biospecimens and interview data were collected from all breast carcinoma cases and their individually matched controls.

RESULTS. Breast carcinoma risk was found to be statistically significantly increased when higher blood levels of C-peptide and IGFs were noted in a dose-response manner. There was a statistically significant twofold to threefold increased risk of breast carcinoma for women in the highest quartile of C-peptide, IGF-1, or IG-FBP-3 compared with women in the lowest quartiles. Women with high levels of both C-peptide and IGF-1 or IGFBP-3 also were found to have a substantially higher risk of breast carcinoma than those women with a high level of only one of these molecules. The adjusted odds ratios (ORs) were 3.79 (95% confidence interval [95% CI], 2.03–7.08) for those with a higher level of both C-peptide and IGF-1 and 4.03 (95% CI, 2.06–7.86) for those with a higher level of both C-peptide and IGFBP-3.

CONCLUSIONS. The results of the current study suggest that insulin resistance and IGFs may synergistically increase the risk of breast carcinoma. *Cancer* **2004;100: 694–700.** © *2004 American Cancer Society.*

KEYWORDS: insulin-like growth factors (IGF), C-peptide, insulin resistance, breast carcinoma.

A recent area of interest in breast carcinoma research is the interplay between insulin resistance and insulin-like growth factors (IGFs) in relation to breast carcinoma risk. Insulin has been shown to have a mitogenic effect in some in vitro systems, inducing a dose-dependent growth response in breast carcinoma cell lines. The IGF family includes the polypeptide ligands IGF-1 and IGF-2, their cognate receptors, six binding proteins (i.e., IGFBP-1 to IGFBP-6), and IGFBP proteases. A large number of in vitro studies have shown that IGFs are strong mitogens for a variety of cancer cells, including many breast carcinoma cell lines.[1] IGFs also are reported to inhibit the cell apoptotic pathway to facilitate cell proliferation.[2,3] The combination of these mitogenic and antiapoptotic effects is reported to have a profound impact on tumor growth.[4-6] IGF-1 and IGF-2 are present in the circulation, in which the majority of them (> 90%) are bound to IGFBP-3.[4,7,8] IGFBPs can inhibit or enhance the action of IGFs, result-

ing in either the suppression or stimulation of cell proliferation depending on the concentration of binding proteins, phosphorylation status, and proteolytic fragmentation.[4,9,10] IGFBP-3 also may promote cell proliferation independently of IGF receptors.[11,12]

Whereas insulin itself is an important growth factor that appears to influence breast carcinoma cells in vitro, it is believed that part of its growth-promoting effect in vivo may be through its role in regulating IGF-1 and IGF-2 production and bioavailability.[6] Cumulative evidence suggests that insulin resistance and high IGF levels interact synergistically to increase a patient's risk of breast carcinoma.[3,13,14] It has been suggested that insulin levels determine the bioavailable level of IGF-1 in the tissues by regulating the production and proteolysis of IGFBP-1.[6,15] IGFs and insulin together have been shown to stimulate motility in human breast carcinoma cell lines, an effect that could enhance migration and invasion.[9,16,17]

Despite evidence from in vitro and in vivo experiments, to our knowledge virtually no epidemiologic studies to date have evaluated the interplay of insulin resistance and IGFs in the etiology of breast carcinoma. There is growing epidemiologic evidence that high circulating levels of IGF-1 are associated with an increased risk of breast carcinoma in women,[18-20] particularly at premenopausal ages.[21-23] The association with IGFBP-3, however, has been inconsistent with regard to findings from previous studies and both positive and inverse associations have been reported.[24,25] The conflicting results from epidemiologic studies are not unfounded, given the dual roles of IGFBP-3 protein in regulating the actions of IGF-1/2. In human studies, insulin resistance often is measured using blood C-peptide, a 31-amino acid peptide that is a byproduct of insulin production.[12] Several studies have shown that blood levels of insulin or C-peptide may be related to the risk of breast carcinoma. We recently reported a positive relation between blood IGF-1 and IGFBP-3[26] and C-peptide[27] levels with breast carcinoma risk in a small ancillary study performed within the Shanghai Breast Cancer study. In the current report, we reevaluated these associations using a larger sample size and explored possible interactions between C-peptide and IGF-1, IGF-2, and IGFBP-3.

MATERIALS AND METHODS

The Shanghai Breast Cancer Study was designed to recruit women ages 25–64 years who were newly diagnosed with breast carcinoma between August 1996 and March 1998, and a group of community controls for a population-based case–control study.[28] All study subjects were permanent residents of urban Shanghai.

They had no prior history of cancer and were alive at the time of the interview. Through a rapid case-ascertainment system, supplemented by the population-based Shanghai Cancer Registry, 1602 eligible breast carcinoma cases were identified during the study period and in-person interviews were completed for 1459 of the eligible cases (91.1%). The controls were selected randomly from female residents in urban Shanghai, using the population-based Shanghai Resident Registry and frequency-matched to cases by age (at 5-year intervals). In-person interviews were completed with 1556 of the 1724 eligible controls identified (90.3%).

Trained interviewers measured each eligible subject for her weight, waist and hip circumference, and sitting and standing heights and conducted an in-person interview according to a standard protocol. A structured questionnaire was used to elicit detailed information concerning demographic factors, menstrual and reproductive history, hormone use, dietary habits, prior disease history, physical activity, tobacco and alcohol use, weight, and family history of cancer. Information regarding usual adult dietary intake was collected using a comprehensive quantitative food frequency questionnaire (FFQ) that covers > 85% of the foods consumed in Shanghai. Blood samples were collected from approximately 83% of the study participants.

An individually matched case–control substudy was built into the Shanghai Breast Cancer Study to increase the comparability of cases and controls in studying quantitative biomarkers. For each case whose samples were collected before any cancer treatment, a control was selected from the pool of subjects who completed the study and individually matched to the index case by age (± 3 years), menopausal status, and date of sample collection (± 30 days). Successful matches were completed for 400 case–control pairs for the current study. To eliminate between-assay variability in case–control comparisons, samples from a matched case–control pair were included in the same batch of assays of blood IGF and C-peptide levels. Three case–control pairs were excluded from all analyses because of laboratory failure with regard to biomarker assays.

Fasting blood samples (10 mL from each woman) were collected in the morning using either ethylenediamine tetraacetic acid (EDTA) or heparin Vacutainer® tubes (Becton Dickinson, San Jose, CA). Immediately after collection, the samples were placed in portable insulated cases with ice pads (4 °C) and transported to the Shanghai Cancer Institute for processing and storage. All samples were aliquoted and stored at -70 °C within 4 hours after collection. Plasma concentrations

TABLE 1
Comparison of Cases and Controls to Demographics and Selected Breast Carcinoma Risk Factors:
The Shanghai Breast Cancer Study, 1996–1998

Characteristics	Cases[a] (n = 397)	Controls[a] (n = 397)	P value[b]
Age (yrs)	47.8 ± 7.8	47.6 ± 7.9	0.20
Education, elementary or lower (%)	12.56	14.61	0.32
Breast cancer in first-degree relatives (%)	1.5	0.75	0.16
Physically active (%)	39.6	35.0	0.005
Body mass index	23.5 ± 3.3	22.9 ± 3.2	0.0186
Age at first live birth (yrs)[c]	26.9 ± 4.1	26.3 ± 3.9	0.01
Menarcheal age (yrs)	14.7 ± 1.7	14.9 ± 1.7	0.11
Menopausal age (yrs)[d]	48.5 ± 4.5	47.8 ± 4.5	0.12
Energy intake (kcal/day)	1905.7 ± 470.3	1862.3 ± 481.9	0.16
Total fat intake (g/day)	37.1 ± 19.3	36.8 ± 15.9	0.79
Total meat intake (g/day)	93.1 ± 69.2	84.3 ± 53.0	0.04
IGF-I[e]	150.6 (144.52–156.92)	138.5 (133.48–143.80)	< 0.001
IGF-II[e]	820.5 (797.38–844.19)	798.6 (779.46–820.64)	0.034
IGFBP-3[e]	3963.9 (3813.56–4119.54)	3718.2 (3586.50–3854.76)	< 0.0001
C-peptide[e]	1.43 (1.34–1.52)	1.19 (1.12–1.26)	< 0.0001

IGF: insulin-like growth factor; IGFBP-3: insulin-like growth factor binding protein-3.

Subjects with missing values were excluded from the analysis.

[a] Unless otherwise specified, the mean ± the standard deviation are presented.

[b] P values were derived from chi-square tests for categoric variables and Student t tests for paired data for continuous variables.

[c] Among women who had live births.

[d] Among postmenopausal women.

[e] The geometric means and 95% confidence intervals are presented.

of IGF-1, IGF-2, and IGFBP-3 were determined using commercially available enzyme-linked immunoadsorbent assay (ELISA) kits (Diagnostic Systems Laboratories, Inc., Webster, TX). The calibrators used in the assays ranged from 4.5–640 ng/mL for IGF-1, 500–2000 ng/mL for IGF-2, and 2.5–100 ng/mL for IGFBP-3. For IGFBP-3 measurement, plasma samples were diluted at 1:100 in an assay buffer. The intraassay and interassay precisions were 1.5–3.4% and 1.5–8.5%, respectively, of coefficient of variation (CV) for IGF-1; 4.2–7.2% and 6.3–10.7%, respectively, of CV for IGF-2; and 0.5–1.9% and 1.8–3.9%, respectively, of CV for IGFBP-3. Each assay had no cross-reaction with other members of the IGF family. Serum C-peptide was measured using an enzymatically amplified, one-step sandwich-type ELISA assay kit (Diagnostic Systems Laboratories, Inc.) and the ELISA assay was performed according to the manufacturer's instructions. Sample aliquots of 20 μL were pipetted into microtiter wells coated with anti-C-peptide antibodies and incubated with 200 μL of a buffered solution of anti-C-peptide antibody conjugated to horseradish peroxidase. Plasma samples were measured in duplicate to improve reliability. These methods were used in the majority of previous epidemiologic studies.[26,27]

Both parametric and nonparametric methods were used to analyze the data collected from the current study. Log-transformed data were used in Student t tests for paired data to compare the mean differences between cases and controls. To evaluate the potential dose-response relation between biomarker level and risk of breast carcinoma, cases and controls were categorized into four groups according to the quartile distribution of the biomarker level among controls. Odds ratios (OR) and 95% confidence intervals (95% CI) for the upper three quartile groups were derived using conditional logistic regression, compared with the lowest quartile group. Multivariate analyses were performed to adjust for potential confounding variables. A score variable was created by summing the quintile level (0, 1, 2, 3, and 4) of C-peptide, IGF-1, and IGFBP-3 and used in the analysis to evaluate the joint association between these three biomarkers and risk of breast carcinoma. The assigned scores ranged from 0–12. Tests for trends were performed in logistic regressions by assigning the score "j" to the "jth" level of the variable selected. All statistical analyses were based on two-tailed probability.

RESULTS
Comparisons of selected demographics and risk factors between breast carcinoma patients and their matched controls are shown in Table 1. There were no statistically significant differences noted between cases and controls with respect to age and education. Although cases had a higher proportion of family his-

TABLE 2
Adjusted ORs and 95% CIs for the Association between Breast Carcinoma and Blood Levels of C-Peptide, IGF-1, IGF-2, and IGFBP-3:
The Shanghai Breast Cancer Study, 1996–1998

	No. of cases	No. of controls	OR (95% CI)[a]	OR (95% CI)[b,c]
IGF-1 (ng/mL)				
Q1 (low)	85	101	1.00	1.00
Q2	77	98	1.19 (0.74–1.92)	1.00 (0.60–1.64)
Q3	91	99	1.37 (0.83–2.26)	1.01 (0.58–1.74)
Q4	145	99	2.38 (1.44–3.93)	1.54 (0.87–2.73)
Trend test			$P < 0.01$	$P = 0.12$
IGF-2 (ng/mL)				
Q1(low)	83	100	1.00	1.00
Q2	99	99	1.36 (0.85–2.17)	0.87 (0.52–1.47)
Q3	94	99	1.79 (1.01–3.18)	0.96 (0.50–1.86)
Q4	122	99	2.47 (1.28–4.75)	0.99 (0.44–2.22)
Trend test			$P = 0.007$	$P = 0.97$
IGFBP-3 (ng/mL)				
Q1 (low)	99	100	1.00	1.00
Q2	57	99	0.74 (0.42–1.32)	0.59 (0.31–1.06)
Q3	98	99	1.40 (0.76–2.57)	0.96 (0.49–1.86)
Q4	144	99	3.45 (1.72–6.93)	2.12 (0.99–4.55)
Trend test			$P < 0.001$	$P = 0.0045$
C-peptide				
Q1 (low)	57	93	1.00	
Q2	85	118	1.32 (0.77–2.30)	
Q3	133	94	2.80 (1.62–4.87)	
Q4	123	92	2.64 (1.47–4.74)	
Trend test			$P < 0.001$	

ORs: odds ratios; 95% CI: 95% confidence interval; IGF: insulin-like growth factor; IGFBP-3: insulin-like growth factor binding protein-3; Q: quartile.
[a] Adjusted for physical activity, age at first live birth, body mass index, and total meat intake.
[b] Adjusted for physical activity, age at first live birth, body mass index, total meat intake, and either insulin-like growth factor binding protein 3 or insulin-like growth factor-1.
[c] Adjusted for physical activity, age at first live birth, body mass index, total meat intake, and both insulin-like growth factor binding protein-3 and insulin-like growth factor-1.

tory, a lower age at menarche, an older age at menopause, and were found to consume more energy and fat, the differences were not found to be statistically significant. Compared with controls, cases were more physically active, had a higher body mass index (BMI), an older age at the first live birth, and were found to consume more meats. Therefore, physical activity, BMI, age at first live birth, and total meat intake were considered potential confounders and adjusted for in some of the subsequent analyses. Cases were found to have significantly higher plasma levels of IGF-1, IGF-2, IGFBP-3, and C-peptide than controls.

Table 2 shows the association between breast carcinoma risk and IGF-1, IGF-2, IGFBP-3, and C-peptide. Because interactions did not exist between IGF-1, IGF-2, and IGFBP-3, mutual adjustment for these variables also was performed. A significant association was noted when IGF-1 was analyzed adjusting for traditional breast carcinoma risk factors (OR, 2.38; 95% CI, 1.44–3.93) (P value for trend = 0.01), but the association became insignificant when adjusting for other IGF molecules. A significant association between high IGFBP-3 and increased breast carcinoma risk was noted in both univariate and multivariate models. The ORs were 3.45 (95% CI, 1.72–6.93) when adjusting for traditional risk factors and 2.23 (95% CI, 0.89–5.56) when IGF-1 was adjusted. The association between C-peptide and breast carcinoma risk was found to be statistically significant; the OR was 2.64 (95% CI, 1.47–4.74) (trend test $P < 0.001$) after adjusting for traditional risk factors. There was no association noted between IGF-2 and risk of breast carcinoma; therefore, IGF-2 was not included in subsequent analyses.

The joint association between breast carcinoma risk and C-peptide and IGF-1 or IGFBP-3 was evaluated in Table 3. The risk of breast carcinoma was found to increase with the blood C-peptide level regardless of the level of IGF-1 or IGFBP-3. Women who had a high level of both C-peptide and IGF-1 or IGFBP-3 were at a particularly increased risk of developing breast carcinoma. However, the interaction between the IGF variables and C-peptide was not found to be statistically significant on the multiplicative scale.

TABLE 3
Joint Associations between Blood Levels of C-Peptide and IGF-1, IGF-2, and IGFBP-3 with the Risk of Breast Carcinoma:
The Shanghai Breast Cancer Study, 1996–1998

| IGF levels (by median) | Serum level of C-peptide by tertile | | | | | |
| | < 0.090 (ng/mL) | | 0.090–1.443 (ng/ml) | | > 1.443 (ng/ml) | |
	Cases/controls	OR (95% CI)	Cases/controls	OR (95% CI)	Cases/controls	OR (95% CI)
IGF-I						
≤ 141 (ng/mL)	44/86	1.00	57/66	1.88 (1.01–3.51)	61/47	2.62 (1.37–5.02)
> 141 (ng/mL)	34/37	2.02 (1.00–4.11)	85/83	2.54 (1.41–4.56)	117/78	3.79 (2.03–7.08)
			P value for interaction, 0.74			
IGFBP-3						
≤ 3741 (ng/mL)	32/58	1.00	46/71	1.04 (0.55–1.98)	78/70	1.38 (0.68–2.80)
> 3741 (ng/mL)	46/65	1.43 (0.76–2.70)	96/78	2.72 (1.45–5.10)	100/55	4.03 (2.06–7.86)
			P value for interaction, 0.19			

IGF: insulin-like growth factor; IGFBP-3: insulin-like growth factor binding protein-3; OR: odds ratio; 95% CI: 95% confidence interval.
[a] Adjusted for physical activity, age at first live birth, body mass index, and total meat intake.

TABLE 4
Combined Association of C-Peptide, IGF-1, and IGFBP-3 Stratified by Menopausal Status with Breast Carcinoma Risk:
The Shanghai Breast Cancer Study, 1996–1998

| Score variable | Score variable of C-peptide, IGF-1, and IGFBP-3 (by quartile) | | | | | |
	Q1 (0–1)	Q2 (2–3)	Q3 (4–5)	Q4 (6–7)	Q5 (8–9)	P value
	All subjects					
Case/control	18/28	58/94	88/127	163/110	71/38	< 0.001
OR (95% CI)	1.00	1.14 (0.55–2.38)	1.25 (0.61–2.59)	2.52 (1.23–5.20)	4.07 (1.74–9.53)	
	Premenopausal women					
Case/control	8/16	31/46	48/84	112/78	51/28	< 0.001
OR (95% CI)	1.00	1.11 (0.37–3.30)	1.12 (0.41–3.10)	2.17 (0.78–6.06)	4.64 (1.44–14.97)	
	Postmenopausal women					
Case/control	10/12	27/48	40/43	51/52	20/10	0.0011
OR (95% CI)	1.00	0.93 (0.30–2.86)	2.04 (0.58–7.14)	3.20 (0.91–11.22)	5.30 (1.13–24.89)	

IGF: insulin-like growth factor; IGFBP-3: insulin-like growth factor binding protein-3; Q: quartile; OR: odds ratio; 95% CI: 95% confidence interval.
[a] Adjusted for physical activity, age at first live birth, body mass index, and total meat intake.

The score variable of C-peptide, IGF-1, and IGFBP-3 was analyzed to evaluate a possible dose-response relation between breast carcinoma risk and a combined exposure level of these molecules, stratified by menopausal status (Table 4). Breast carcinoma risk was found to be strongly associated with the index; the highest risk was observed among women with the highest score for all 3 molecules, (OR, 4.07; 95% CI, 1.74–9.53). This association was found to be similar for both premenopausal and postmenopausal women.

DISCUSSION

In a previous small study of 143 case-control participants, we reported that the blood C-peptide level was positively associated with breast carcinoma risk.[27] The results of the current study, a larger study of 398 case-control pairs, confirm this finding. These results are supported by several previous epidemiologic studies. A case-control study in Amsterdam showed that the serum level of C-peptide was related positively to the risk of breast carcinoma and that this association was independent of general adiposity or abdominal obesity.[19] Muti et al. reported a modest association between higher fasting insulin levels and breast carcinoma risk in a nested case-control study.[23] Fasting insulin was examined in a cohort of cases with early-stage breast carcinoma and was found to be positively associated with a threefold increased risk of death and a twofold increased risk of recurrence in both premenopausal and postmenopausal women.[29] In a small

case–control study of 45 postmenopausal breast carcinoma cases, blood levels of C-peptide, fasting insulin, and proinsulin were found to be somewhat higher in cases compared with controls, but the differences were not found to be statistically significant.[30] A nested case–control study conducted in New York City showed no association between risk of breast carcinoma and nonfasting levels of serum C-peptide.[22] Two prospective studies could not detect a relation between nonfasting C-peptide levels and postmenopausal breast carcinoma.[25,31]

The studies cited above used various biomarkers such as proinsulin as a proxy measure of insulin levels. The current study used serum C-peptide from a fasting blood sample, which to our knowledge is a more accurate measure of insulin levels. C-peptide is a byproduct of insulin production. The major advantages of measuring fasting serum C-peptide levels over insulin levels are twofold. First is the ability to readily distinguish endogenous insulin levels in the presence of exogenous insulin administration. Second, C-peptide can be measured in the presence of circulating insulin antibodies, which develop in most diabetic patients who have been treated with insulin injections for longer than a few weeks. Typically, circulating insulin antibodies interfere with the usual immunoassay for insulin. In this instance, these antibodies do not cross-react with human C-peptide.[32]

Similar to the results of the current study, blood IGF-1 has been found fairly consistently to be positively associated with the risk of breast carcinoma. We also found levels of circulating IGFBP-3 to be elevated in women with breast carcinoma, which is congruent with other case–control studies.[33] However, in contrast to the findings of the current study regarding circulating IGFBP-3 (adjusted for IGF-1) are previous case–control studies in which there were inverse[19] and null associations.[20] Prospective studies also have yielded inconsistent results with regard to circulating IGFBP-3. Some studies have shown a positive association in premenopausal women only[24] or in both premenopausal and postmenopausal women,[23,28] whereas other studies reported an inverse association in premenopausal women age < 50 years[21] or in postmenopausal women.[24] Null associations also have been reported.[22,25] The conflicting results from epidemiologic studies are not unexpected, given the dual roles of IGFBP-3 protein in regulating the actions of IGF-1/IGF-2. In addition, blood levels of IGFBP-3 may not reflect the level of this protein in the target tissues, and to our knowledge, the majority of epidemiologic studies have no access to normal target tissue samples with which to evaluate the association between this protein and cancer risk.

Despite many studies concerning IGFs and C-peptide singly,[19,22,23,28,30] no study published to date has evaluated the joint effect of these biomarkers on the risk of breast carcinoma. The dearth of literature in this area may be attributed to the use of a small sample size in which the statistical power was inadequate to evaluate the joint effects of multiple factors and blood samples from cancer cases collected after cancer treatment. These limitations would preclude studies that examine how insulin resistance and IGF activation are involved in breast malignancies. Signaling pathways involved in breast carcinoma cell growth are not to our knowledge simple or linear. Numerous divergent and similar pathways are stimulated after insulin-like growth factor receptor 1 (IGF-IR) activation, which then encroach on multiple other pathways that relate to the biology of breast carcinoma. The current study findings of a potentially synergistic effect of C-peptide and IGFs are consistent with the results from in vitro experiments showing the interplay of these molecules in the etiology of breast carcinoma.

As in any case–control study, the use of biologic samples collected after disease diagnosis for evaluating the association between biomarkers of interest with disease risk is a major concern in the current study. However, blood samples for this study were collected prior to any cancer treatment, and the majority of breast carcinoma patients were diagnosed at early stages of disease. Because samples from most breast carcinoma patients were collected within days of the preliminary diagnosis of breast carcinoma, the lifestyle changes in a such short interval should not be appreciable, particularly for those patients with early-stage breast carcinoma. We further analyzed the association by disease stage of the cancer diagnosis and found that associations were somewhat stronger for early-stage breast carcinoma cases, indicating that the observed positive association is unlikely to be the consequence of tumor growth in breast carcinoma patients.

The current population-based case–control study conducted in Chinese women from Shanghai revealed a potential synergistic effect of C-peptide and IGF-1 or IGFBP-3 on the risk of breast carcinoma. These findings are biologically plausible and supported by studies in cell cultures suggesting the interactive effects of these molecules. Further studies with prospectively collected biologic samples are warranted to evaluate this association further.

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Appendix 6: Abstract and corresponding poster presentation entitled "Energy Balance; C-peptide, Insulin–Like Growth Factors and Breast Cancer Risk" at NAASO/ADA, 2003.

Appendix 7: Abstract and corresponding poster presentation entitled "Combined Association of Energy Balance, Lifestyle Factors and Breast Cancer Risk" for poster presentation at the 95th annual AACR meeting, 2004.

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Appendix 8: Malin et al, (2005) Energy Balance and Breast Cancer Risk. Cancer Epidemiol Biomarkers Prev;14(6) 1496-1501.

Energy Balance and Breast Cancer Risk

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Abstract

We evaluated the hypothesis that a pattern of behavioral exposures indicating positive energy balance [i.e., less exercise/sport activity, high body mass index (BMI), or high energy intake] would be associated with an increased breast cancer risk in the Shanghai Breast Cancer Study, a population-based study of 1,459 incident breast cancer cases and 1,556 age frequency-matched controls. Participants completed in-person interviews that collected information on breast cancer risk factors, usual dietary intake and physical activity in adulthood. Anthropometric indices were measured. Odds ratios and 95% confidence intervals were estimated by logistic regression to describe the individual and joint effects of the exposures on breast cancer risk. Lack of exercise/sport activity, low occupational activity, and high BMI were all individually associated with increased risk of breast cancer [odds ratios (OR) ranged from 1.49 to 1.86]. In general, women with lower exercise/sport activity level and higher BMI, or those with higher energy intake, were at an increased risk compared with women who reported more exercise/sport activities, had lower BMIs, or reported less energy intake. There was a significant multiplicative interaction ($P = 0.02$) between adult exercise/sport activity and BMI, with inactive women in the upper BMI quartile being at increased risk (OR, 2.16; 95% confidence interval, 1.25-3.74) compared with their active and lean counterparts. This association was stronger in postmenopausal than in premenopausal women, and nonexercising postmenopausal women with higher BMIs were at substantially increased risk (OR, 4.74; 95% confidence interval, 2.05-12.20). Our study suggests that promotion of behavior patterns that optimize energy balance (weight control and increasing physical activity) may be a viable option for breast cancer prevention. (Cancer Epidemiol Biomarkers Prev 2005;14(6):1496–501)

Introduction

Restriction of calories by 10% to 40% of *ad libitum* intake inhibits mammary gland tumors in animal models by decreasing cell proliferation, increasing apoptosis, and possibly through antiangiogenic processes (1, 2). The effect of energy restriction on breast cancer risk has been examined in epidemiologic studies with mixed results. Michels et al. (3) prospectively followed a cohort of Swedish women diagnosed and treated for anorexia before age 40 and reported they had nearly half the risk of breast cancer compared with age-matched controls. Studies examining the influence of war-related famine on breast cancer have provided conflicting results with one study suggesting decreased risk (4), and another study suggesting increased risk (5), for women exposed to short-lived famine conditions. In contrast, premenopausal obesity is associated with reduced risk of the disease, whereas postmenopausal obesity is associated with increased risk (6). Long-term participation in high levels of physical activity has also been associated with reduced risk of the disease (7, 8). The independent effect of energy intake on breast cancer risk has been difficult to estimate because body size and physical activity are strong determinants of total energy expenditure (9).

Given the substantial level of weight gain in industrialized countries in the last two decades (10, 11), there is great interest in understanding the influence of energy balance on cancer risk and to develop preventive strategies that can effectively minimize excess risk. The potent anticancer effect of caloric restriction in animals is clear, but caloric restriction alone is not generally considered to be a feasible strategy for cancer prevention in humans. However, the identification and development of preventive strategies that "mimic" the anticancer effects of low energy intake are desirable (12). Caloric restriction limits systemic energy availability by restricting energy intake at a low level (1). Energy balance can be conceptualized as the difference between intake and expenditure and, thus, can be modulated by changes in intake, expenditure, or both (1, 13). Acute restriction of available energy in women, by dietary restriction, physical activity energy expenditure, or combinations of both, has been shown to produce a hormonal and metabolic milieu that mimics several features of caloric restriction and is consistent with a low risk for breast cancer (e.g., reduced insulin, less bioavailable insulin-like growth factor-I; refs. 12, 14-17).

A methodologic problem in measuring energy balance, in particular energy intake, is the phenomena of subjects reporting lower energy intake than physiologically required, noted as underreporting (18). Underreporting of energy intake is expressed as a ratio of reported energy intake to estimated basal metabolic rate (EI/BMR_{est}; refs. 19, 20).

Serious underreporting of energy intake has mostly been observed in obese people (21-23). A higher body mass index (BMI) has been shown to be an independent predictor of low EI/BMR_{est} (22, 24, 25). Underreporting energy intake in the obese population may be perpetuated by desire for weight change and level of dietary consciousness. Several studies suggest that obese underreporters are more likely to estimate low intakes of foods perceived unhealthy than those perceived as healthy (25, 26). Systematic underreporting differentiates across members of the study sample and may lead to bias in associations between food intake patterns and certain disease

outcomes; in this case, associations between dietary intakes and obesity-related diseases like breast cancer.

There is limited research on underreporting and increased body size in the Asian population, primarily due to the low prevalence of obesity in the Asian population. Study results have been contradictory to the findings in other world populations where obese individuals are more likely to under-report. A recent study in Japanese female college students where 95% were classified as nonobese (BMI <25 kg/m^2) revealed BMI, body weight, and basal metabolic rate decreased significantly with the increase in EI/BMR$_{est}$ ($P < 0.001$; ref. 27). In short, underreporting of energy intake was predominant in this relatively lean population.

Obesity also has a low prevalence in the Shanghai female population (28). To prevent underreporting, which is indicative in members of the female nonobese Asian population, the instrument used to assess energy intake was an interview administered in-person using a quantitative food frequency questionnaire, based on data from a 24-hour dietary survey in a validation study conducted in 200 Shanghai women (29) versus a self-administered, self-reported food frequency questionnaire. Self-reported dietary questionnaires have been proven to be prone to underestimated energy intake (21, 30).

We have previously reported significant associations of body size (weight, height) and weight gain (28), and high levels of exercise/sport activity (31) with breast cancer risk in the Shanghai Breast Cancer Study. In this report, we reevaluate indicators of energy balance that we have found previously to be associated with risk, as well as energy intake, and test the hypothesis that behavioral patterns indicating greater energy availability (i.e., low exercise/sport activity, high BMI, or high energy intake) may be associated with increased breast cancer risk compared with patterns of exposure indicating restricted energy availability (i.e., high exercise/sport activity and low BMI or low energy intake).

Materials and Methods

The Shanghai Breast Cancer Study was designed to recruit women ages 25 to 64 who were newly diagnosed with breast cancer, and a random sample of healthy controls. Study participants were permanent residents of urban Shanghai and were enrolled in the study between August 1996 and March 1998. Women with a prior history of cancer or who were deceased at the time of interview were not eligible for the study. Through a rapid case ascertainment system, supplemented by the population-based Shanghai Cancer Registry, 1,602 eligible incident breast cancer cases were identified during the study period and in-person interviews were completed for 1,459 (91.1%) of them. Controls were randomly selected from female residents in urban Shanghai, using the population-based Shanghai Resident Registry, and frequency-matched to cases by age (5-year intervals). In-person interviews were completed with 1,556 (90.3%) of 1,724 eligible controls.

Trained interviewers conducted an in-person interview and measured eligible women for weight, circumference of waist and hips, and height (according to a standard protocol; ref. 28). From these data, waist/hip ratio and BMI (kg/m^2) were calculated. The structured questionnaire elicited detailed information on demographic factors, menstrual and reproductive history, hormone use, dietary habits, prior disease history, physical activity, tobacco and alcohol use, and family history of cancer.

Usual adult dietary intake was collected using a comprehensive quantitative food frequency questionnaire that covers >85% of commonly consumed foods in urban Shanghai (32). A physical activity questionnaire assessed exercise/sport activity and occupational activity levels. Women could report up to

five exercises or sport activities during adolescence (13-19 years) and adulthood (last 10 years). Quantitative exercise/sport data were summarized in terms of intensity [metabolic equivalents (MET), duration (h/wk), years of participation, and average energy expenditure during the period (MET-h/wk/y) using standard methods; ref. 31]. Women also reported their occupational physical activity levels for jobs they held for at least 3 years during their lifetime. For each occupation, participants reported the average time spent in "standing or walking" and classified the physical exertion of the job according to three activity categories (i.e., heavy, medium, or nonphysical work). Summary measures were calculated by multiplying the years spent in each occupation by the specific activity variable and then summing the results over all jobs. A similar version of this physical activity questionnaire has been found to be a reliable and valid assessment of exercise and sports in Shanghai women (32).

P values were derived from t tests for univariate comparisons (Table 1). Unconditional logistic regression was used to derive adjusted odds ratios (OR) and 95% confidence intervals (95% CI) to estimate the associations between breast cancer risk and the individual indicators of energy balance (exercise/sport activity, occupational activity, BMI, energy intake) as well as the joint effects of these exposures. Multivariate analyses were adjusted for the following variables: breast cancer in first-degree relative, history of breast fibroadenoma, household income, education, age at first live birth, height, and menopausal status when appropriate. Exposure levels for continuous variables, BMI, and energy intake were categorized according to quartile distributions among controls. Trend tests were conducted by treating categorical variables as the ordinal values of the quartile levels in the model. Multiplicative interactions were evaluated using the cross-product terms of relevant exposures along with the main effect terms in the model.

Results

The descriptive characteristics for cases and controls in the Shanghai Breast Cancer Study are presented in Table 1. Cases had an earlier age of menarche, later age of menopause, and first live birth. Proportionally more cases had breast fibroadenomas and first-degree relatives with breast cancer. There were positive associations between breast cancer risk and low levels of exercise/sport activity in adulthood and lifetime occupational activity, and increased BMI, waist/hip ratio, and

Table 1. Comparisons of cases and controls on demographics and selected breast cancer risk factors (Shanghai Breast Cancer Study, 1996-1999)

Characteristics	Cases ($n = 1,459$)	Controls ($n = 1,556$)	P
Age	47.93 ± 7.99	47.25 ± 8.79	0.24
Education(%)			
No formal education	3.63	5.46	
Elementary school	8.50	8.42	
Middle + high school	74.3	75.4	
Profession, college and above	13.6	10.7	0.012
Breast cancer in first-degree relatives (%)	3.7	2.4	0.045
Ever had breast fibroadenoma (%)	9.6	5.0	<0.001
Nulliparous (%)	5.1	3.9	0.126
Age at first live birth (y)	26.7 ± 4.2	26.2 ± 3.9	<0.01
Menarcheal age (y)	14.5 ± 1.6	14.7 ± 1.7	<0.01
Postmenopausal (%)	34.5	36.2	0.321
Menopausal age (y)*	48.1 ± 4.6	47.4 ± 4.9	0.02

*Among postmenopausal women only.

adult height (Table 2). Greater energy intake alone was not associated with risk of breast cancer (Table 2).

The joint associations of these energy balance indicators reflecting gradations in energy availability with breast cancer are presented for all women and by menopausal status in Table 3. In general, women with combined patterns of exposure indicating a positive energy balance were at increased risk compare to women with exposure patterns indicating more restricted energy balance. That is, women with lower physical activity levels and higher BMIs, and low physical activity levels and high energy intakes, were at increased risk relative to women with higher activity levels who were lean and/or who reported consuming fewer calories (Table 3). A significant multiplicative interaction was found between exercise/sports activity and BMI (Table 3). Women reporting no exercise with BMIs >25 kg/m^2 were at >2-fold increased risk compared with women reporting >2.92 MET-h/d/y of exercise with a BMI of <21 kg/m^2. Higher BMI was unrelated to breast cancer risk among women who exercised (OR, 0.75; 95% CI, 0.38-1.47). Similarly, lack of exercise was unrelated to risk among lean women (OR, 1.29; 95% CI, 0.74-2.23).

The results revealed that the type of physical inactivity pattern differed markedly between premenopausal and postmenopausal women. Among premenopausal women, lack of exercise/sport activity is not associated with risk (OR, 1.11; 95% CI, 0.52-2.38), whereas low levels of occupational activity were associated with increased risk (OR, 2.21; 95% CI, 1.20-4.20).

In contrast, low levels of both exercise/sport activity (OR, 4.74; 95% CI, 2.00-11.19) and occupational activity (OR, 3.13; 95% CI, 1.43-6.86) were associated with increased risk in postmenopausal women. This divergence between menopausal statuses may be explained by younger women engaging in more occupational physical activity and postmenopausal women (22%) engaging in more exercise participation than their younger counterparts (13%).

Table 2. ORs and 95% CIs for breast cancer risk associated with exercise/sport activity, occupational activity, BMI, waist/hip ratio, height and energy intake

	Case/control	OR (95% CI)
Adult exercise/sports (MET-h/d/y)		
>2.92	11/18	1.0
0 < h ≤2.92	147/188	1.33 (0.96-1.83)
0	1,199/1,181	1.86 (1.44-2.41)
P_{trend}	<0.001	
Occupational activity (hours of standing work per day, h/d/y) Adult		
>4	469/581	1.0
0 < h ≤4	716/742	1.17 (0.99-1.37)
0	256/211	1.49 (1.19-1.86)
P_{trend}	0.0006	
BMI (kg/m^2)		
<21	333/439	1.0
21 <BMI <25	679/714	1.27 (1.06-1.52)
>25	443/402	1.49 (1.21-1.83)
P_{trend}	0.0002	
Waist/hip ratio		
<0.76	256/347	1.0
0.76 < waist/hip ratio <0.84	806/868	1.25 (1.03-1.51)
>0.84	394/340	1.60 (1.28-2.00)
P_{trend}	<0.0001	
Energy intake (kcal/d)		
<1,540	355/397	1.0
1,540 < kcal <2,107	716/790	0.99 (0.83-1.18)
>2,107	388/369	1.15 (0.93-1.41)
P_{trend}	0.1921	

NOTE: Data were adjusted for age at interview, education, income, history of fibroadenoma, history of breast cancer among first-degree relatives, and ever had live birth. Occupational activity was measured as the average time spent in standing or walking and classified into one of three activity categories (i.e., heavy, medium, or nonphysical work).

There was no indication of a joint effect between physical activity and energy intake on breast cancer risk among premenopausal women. This finding may reflect the much greater degree of measurement error using an in-person interview to measure food frequency intake than using measured weight and height used to calculate BMI. Joint presence of high-energy intake and low physical activity, however, was associated with a higher risk of breast cancer than each of these factors alone among postmenopausal women. However, test for multiplicative interaction were not statistically significant (Table 3).

Additional analyses were conducted to evaluate the three-way interactions between physical activity, energy intake, and BMI (Table 4). Highest risk was consistently observed for women who were less active, who were heavier, and who consumed more calories. High energy intake added minimal additional risk (Table 4).

Discussion

Few epidemiologic studies of breast cancer have examined the joint effects of physical activity, body size, and energy intake on risk. Those studies that examine the effects of physical activity and body size provide inconclusive evidence on breast cancer risk. Some studies have suggested that higher levels of activity conferred the most benefit among lean postmenopausal (33) versus lean premenopausal women (34). On the other hand, some studies found that higher levels of physical activity are unrelated to postmenopausal (35) and premenopausal women's breast cancer risk (36, 37). In contrast, we found that increased breast cancer risk was associated with occupational inactivity in the higher BMI premenopausal population and with exercise/sport inactivity in the higher BMI postmenopausal population.

Several explanations might explain a difference in the strength of association between physical activity, body size, and risk of breast cancer depending on menopausal status in our study. Discrepancies in findings between studies may be attributed to lack of information about total or specific components of physical activity (thus, no information on occupational activity) in contrast to our study including both exercise/sport and occupational activity. This may explain why there are null findings on physical activity and premenopausal breast cancer risk (36, 37) and limited evidence that a lack of occupational activity is detrimental in the postmenopausal population (33, 35). Our study computed the intensity [metabolic equivalents (MET), duration (h/wk), years of participation, and average energy expenditure during the period (MET-h/wk/y)] using standard methods allowing study of dose-response relationships and long-term effects of physical activity on breast cancer risk. This is in contrast to the Norwegian study that measured the total physical activity (recreational and occupational) using self-administered questionnaires (34) but did not measure the duration and intensity of the activities done.

The joint relationship between higher energy intake and breast cancer risk is less striking in this population. We have taken precautions to minimize underreporting and misclassification bias of energy intake by using a validated in-person food frequency questionnaire versus 24-hour food records that may attenuate the associations between diet and disease. However, individual dietary habits are influenced by a host of social, cultural, customary, and economic factors; thus, assessments of diet in a relatively homogenous population may weaken the disease-diet relationship (38).

There is good evidence that restricted energy availability is associated with a hormonal and/or metabolic milieu that would be predicted to reduce breast cancer risk (16, 39). For example, Loucks et al. (16) report that restricted energy

availability was associated with reduced insulin and bioavailable insulin-like growth factor-I (e.g., insulin-like growth factor-I/insulin-like growth factor binding protein-3 ratio), as well as reduced leptin levels. C peptide and some elements of the insulin-like growth factor axis have been associated with breast cancer risk in the Shanghai Breast Cancer Study (40, 41) as well as a number of other breast cancer studies (42). Leptin levels have been positively associated with cell proliferation in mammary tissue and, thus, may be a growth factor that influences carcinogenesis (43, 44). Moreover, lower levels of energy availability have been associated with luteal phase deficiency (i.e., shorter luteal phase, lower progesterone) in premenopausal women (39). Among postmenopausal women, this exposure pattern would limit the accumulation of adipose tissue during adulthood and subsequently reduce postmenopausal estrogen exposure through aromatization of adrenal androgens (45).

Studies in animal models examining the combined effects of exercise and caloric restriction on cancer outcomes have provided mixed results. Holloszy (46) reported that both exercise and caloric restriction, and the combination of both, reduced cancer mortality. Kritchevsky (47) reported that these same exposures reduced tumor incidence and multiplicity for dimethylhydrazine-induced colon tumors in rats. In contrast, Gillette (48) failed to find evidence of an energy availability effect on 1-methyl-1-nitorsomethylurea–induced mammary tumors in rats. Clearly, more research is needed to elucidate the impact of patterns of behavior that favor lower levels of energy availability on cancer biomarkers and frank cancer outcomes.

Although we believe that these data are consistent with the energy availability hypothesis, we acknowledge some apparent inconsistencies in our results. The type of physical activity that conferred benefit was not consistent for premenopausal and postmenopausal women. Among premenopausal women, low levels of occupational activity were associated with increased risk, whereas among postmenopausal women low levels of both exercise/sport activity and occupational activity were associated with increased risk. This inconsistency may be explained by the lower prevalence of exercise/sport participation among premenopausal compared with postmenopausal women in these data, and because occupational physical activity contributes a greater proportion of overall physical activity energy expenditure in younger women. The discrepancies may also affect the opposite effect of BMI for premenopausal and postmenopausal breast cancer with energy intake. Interestingly, in this study, we also found that the joint effect of physical activity and energy intake was more evident among postmenopausal women, suggesting that energy balance may have differential effects on premenopausal and postmenopausal women.

This study has several strengths. It was a population-based, case-control study that included incident cases and that obtained detailed information about traditional breast cancer risk factors that allowed for full adjustment for possible confounding factors. Participation rates were high (>90%) for both cases and controls, suggesting that the potential for selection bias in this study is low. The primary instruments used to obtain physical activity and dietary information in this study have been tested for reliability and validity in a

Table 3. Association of adult exercise/sport activity levels, occupational activity levels, BMI, energy intake and breast cancer risk between premenopausal and postmenopausal women (Shanghai Breast Cancer Study, 1996-1999)

	BMI (kg/m²)			Energy intake (kcal/d)		
	Q1 (<21)	Q2-Q3 (21 < BMI < 25)	Q4 (>25)	Q1 (<1,540)	Q2-Q3 (1,540 < EI < 2,107)	Q4 (>2,107)
All women						
Adult exercise/sports (MET-h/d/y)						
>2.92	1.0	1.07 (0.57-2.00)	0.75 (0.38-1.47)	1.0	0.83 (0.44-1.56)	0.85 (0.43-1.69)
0 < h ≤ 2.92	1.02 (0.51-2.05)	1.22 (0.66-2.26)	1.42 (0.74-2.74)	1.06 (0.53-2.14)	1.24 (0.67-2.31)	1.05 (0.54-2.08)
0	1.29 (0.74-2.23)	1.68 (0.98-2.88)	2.16 (1.25-3.74)	1.52 (0.87-2.66)	1.49 (0.86-2.59)	1.87 (1.06-3.28)
$P_{interaction}$	0.02			0.31		
Occupational activity (h/d/y)						
>4	1.0	1.13 (0.82-1.56)	1.37 (0.97-1.94)	1.0	1.01 (0.75-1.38)	1.13 (0.80-1.59)
0 < h ≤4	1.01 (0.72-1.41)	1.41 (1.04-1.92)	1.59 (1.13-2.23)	1.16 (0.84-1.62)	1.15 (0.86-1.54)	1.35 (0.97-1.88)
0	1.34 (0.86-2.09)	1.73 (1.19-2.54)	2.47 (1.57-3.88)	1.63 (1.06-2.50)	1.46 (1.01-2.11)	1.82 (1.15-2.88)
$P_{interaction}$	0.35			0.95		
Premenopausal						
Adult exercise/sports (MET-h/d/y)						
>2.92	1.0	0.82 (0.33-2.03)	0.74 (0.24-2.30)	1.0	0.60 (0.19-1.88)	0.58 (0.18-1.89)
0 < h ≤ 2.92	0.65 (0.26-1.62)	0.85 (0.36-2.00)	0.54 (0.20-1.48)	0.56 (0.18-1.78)	0.57 (0.19-1.68)	0.49 (0.15-1.54)
0	0.81 (0.38-1.71)	1.01 (0.48-2.12)	1.11 (0.52-2.38)	0.77 (0.28-2.10)	0.67 (0.25-1.81)	0.87 (0.32-2.39)
$P_{interaction}$	0.17			0.37		
Occupational activity (h/d/y)						
>4	1.0	1.14 (0.79-1.65)	1.09 (0.71-1.68)	1.0	0.98 (0.67-1.44)	1.20 (0.77-1.87)
0 < h ≤ 4	0.94 (0.65-1.38)	1.29 (0.91-1.83)	1.31 (0.87-1.98)	1.24 (0.82-1.86)	1.01 (0.70-1.46)	1.30 (0.85-1.97)
0	1.55 (0.93-2.58)	1.78 (1.13-2.80)	2.25 (1.20-4.20)	1.92 (1.12-3.29)	1.57 (0.99-2.51)	1.71 (0.92-3.19)
$P_{interaction}$	0.44			0.37		
Postmenopausal						
Adult exercise/sports (MET-h/d/y)						
>2.92	1.0	1.65 (0.65-4.19)	1.19 (0.45-3.12)	1.0	0.96 (0.43-2.13)	0.90 (0.37-2.19)
0 < h ≤ 2.92	1.68 (0.53-5.34)	1.80 (0.69-4.68)	3.30 (1.27-8.57)	1.32 (0.51-3.43)	1.85 (0.83-4.13)	1.55 (0.64-3.77)
0	2.07 (0.85-5.07)	3.00 (1.28-7.05)	4.74 (2.00-11.19)	1.82 (0.87-3.79)	2.51 (1.24-5.08)	2.71 (1.31-5.63)
$P_{interaction}$	0.12			0.34		
Occupational activity (h/d/y)						
>4	1.0	1.14 (0.57-2.28)	1.94 (0.97-3.88)	1.0	1.11 (0.66-1.85)	1.01 (0.57-1.78)
0 < h ≤ 4	1.26 (0.60-2.63)	1.81 (0.92-3.57)	2.17 (1.09-4.32)	1.01 (0.56-1.81)	1.51 (0.92-2.49)	1.45 (0.84-2.51)
0	0.97 (0.38-2.51)	1.83 (0.85-3.94)	3.13 (1.43-6.86)	1.25 (0.60-2.59)	1.36 (0.74-2.50)	2.06 (1.04-4.08)
$P_{interaction}$	0.64			0.34		

NOTE: Data were adjusted for age at interview, education, income, history of fibroadenoma, history of breast cancer among first-degree relatives, ever had live birth, height, and menopausal status. Occupational activity was measured as the average time spent in standing or walking and classified into one of three activity categories (i.e., heavy, medium, or nonphysical work).

Table 4. Joint effects of adult exercise and sport activity levels, occupational activity levels, energy intake, and BMI on breast cancer risk

	Energy intake (kcal/d)		BMI (kg/m^2)	
		Low (<21)	Intermediate (21 $\leq$ BMI $\leq$ 25)	High ($\geq$25)
All women				
Adult exercise/sports (MET-h/d/y)				
Yes	Low (<1,794)	1.0 (Reference)	0.94 (0.52-1.71)	0.92 (0.48-1.76)
	High (>1,794)	0.74 (0.37-1.46)	0.91 (0.51-1.63)	0.81 (0.44-1.50)
No	Low (<1,794)	1.12 (0.66-1.90)	1.25 (0.75-2.09)	1.69 (0.98-2.89)
	High (>1,794)	0.93 (0.54-1.60)	1.47 (0.88-2.46)	1.79 (1.06-3.05)
$P_{interaction}$		0.65		
Premenopausal				
Occupational activity (h/d/y)				
Yes	Low (<1,794)	1.0 (Reference)	1.12 (0.83-1.52)	1.08 (0.72-1.61)
	High (>1,794)	0.85 (0.59-1.21)	1.23 (0.90-1.68)	1.22 (0.85-1.75)
No	Low (<1,794)	1.57 (0.88-2.79)	1.48 (0.88-2.51)	2.82 (1.29-6.18)
	High (>1,794)	1.30 (0.64-2.65)	1.99 (1.13-3.53)	1.51 (0.64-3.54)
$P_{interaction}$		0.14		
Postmenopausal				
Adult exercise/sports (MET-h/d/y)				
Yes	Low (<1,794)	1.0 (Reference)	0.90 (0.35-2.31)	1.15 (0.45-2.77)
	High (>1,794)	0.59 (0.19-1.84)	1.03 (0.43-2.51)	1.12 (0.45-2.77)
No	Low (<1,794)	1.36 (0.55-3.40)	1.59 (0.68-3.73)	2.55 (1.07-6.04)
	High (>1,794)	1.01 (0.39-2.58)	1.83 (0.79-4.25)	2.84 (1.21-6.67)
$P_{interaction}$		0.21		

NOTE: Data were adjusted for age at interview, education, income, history of fibroadenoma, history of breast cancer among first-degree relatives, menopausal status, height, and ever had live birth. Occupational activity was measured as the average time spent in standing or walking and classified into one of three activity categories (i.e., heavy, medium, or nonphysical work).

population of women from Shanghai, and both were found to be reliable and valid instruments for stratifying women by physical activity and energy intake levels (32, 49). Another strength, distinct from other epidemiologic studies, is that BMI was calculated from measured rather than self-reported weight and height within days of cancer diagnosis, thus reducing measurement errors and some of the effects of therapy on body weight. In summary, we observed that women with higher levels of energy availability, by virtue of their low physical activity and high BMI or energy intake levels, were at an increased breast cancer risk compared with women with more restricted energy availability. These findings support current breast cancer prevention efforts that seek to increase physical activity levels and minimize age-related weight gain (50, 51). The relationship between these patterns of exposure modifying breast cancer risk and underlying biological mechanisms remains indeterminate, yet our findings are consistent with evidence that restricted energy availability can induce hormonal and metabolic changes that are consistent with reduced breast cancer risk. The fact that many of these pathways overlap with mechanisms proposed to explain the anticancer effect of caloric restriction suggests that balancing energy expenditure with intake may mimic some of the elements of caloric restriction.

Additional research is needed to document similar relationships in other study populations and to better understand the mechanisms underlying associations observed in this report. Further health promotion programs should be created to reduce the prevalence of overweight and sedentary lifestyle, two modifiable risk factors for breast cancer risk, and many other health risks for women.

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REPORTS

Insulin-Like Growth Factor-I, Soy Protein Intake, and Breast Cancer Risk

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Abstract: Previous studies have found that estrogen enhances the effect of insulin-like growth factor-I (IGF-I) levels on breast cancer cell growth. Participants in the Shanghai Breast Cancer Study (SBCS) consumed large amounts of soy that was high in isoflavones, which act as weak estrogens and as anti-estrogens. We assessed whether soy protein intake modified the effect of IGF-I levels on breast cancer risk. The SBCS is a population-based case-control study of breast cancer among women aged 25–64 conducted between 1996 and 1998 in urban Shanghai. In-person interviews were completed with 1,459 incident breast cancer cases ascertained through a population-based cancer registry and 1,556 controls randomly selected from the general population (with respective response rates of 91% and 90%). This analysis is restricted to the 397 cases and 397 matched controls for whom information on IGF-I levels was available. For premenopausal breast cancer, we found nearly significant interactions between soy protein intake and IGF-I levels (P = 0.080) and insulin-like growth factor-binding protein-3 (IGFBP-3) levels (P = 0.057). The direction of the interaction appeared to be negative for IGF-I levels but was positive for IGFBP-3 levels. No interaction was evident between soy protein intake and IGF-I or IGFBP-3 levels among postmenopausal women. Our results suggest that soy protein intake may negatively modulate the effect of IGF-I and may positively modulate the effect of IGFBP-3 levels on premenopausal breast cancer risk. Further studies are needed to confirm our finding and to understand the biological mechanisms of these potential interactions.

Introduction

Insulin-like growth factor-I (IGF-I) is thought to play a role in the pathogenesis of breast cancer due to its mitogenic and anti-apoptotic effects on mammary cell lines (1). Insulin-like growth factor-binding protein-3 (IGFBP-3) regulates IGF-I bioactivity by binding to IGF-I (2). Of the nine human studies of IGF-I levels and premenopausal breast cancer (3–11), IGF-I was positively associated in four studies of Caucasian women (3–6) and in the Shanghai Breast Cancer Study (SBCS) of Asian women (7). Seven of these studies also investigated IGFBP-3, with four studies reporting positive associations (3,5–7) and three studies reporting no association (8,9,11). Only one human study, conducted among African-American women (12), of the 12 studies of IGF-I levels and postmenopausal breast cancer (4–7,9–11,13–16) found a positive relation. Similarly, only one study, using the SBCS (7), of the eight studies of IGFBP-3 and breast cancer (5,6,9,11,14–16) reported an elevated risk of postmenopausal breast cancer associated with increased IGFBP-3. In vitro studies have shown that estrogen enhances the effect of IGF-I on breast cancer cell growth (17,18), and thus the association of IGF-I with breast cancer risk may be modified by estrogens. One in vivo study, using the SBCS, investigated whether estrogen modified the effect of IGF-I on breast cancer risk (19). They reported synergistic effects between IGF-I levels and two estrogen-related hormones, estrone and testosterone, on breast cancer risk among women diagnosed premenopausally and postmenopausally.

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High consumption of soy during childhood and adulthood has been hypothesized to be protective against breast cancer. One of several mechanisms proposed for this effect is its richness in isoflavones, which may reduce estrogen activity in the breast by competing as weak estrogens for receptor sites (20). Isoflavones may also reduce estrogen synthesis (21) and increase sex hormone-binding globulin (22). Of the 12 human studies of adult soy intake on breast cancer risk (23–34) only 4 of those conducted among Asian or Asian-American populations, who consume large amounts of soy, have found statistically significant inverse associations (23–26). A recent Japanese cohort study identified significant inverse relations for isoflavones and breast cancer risk, especially among postmenopausal women, but not for soy in general (35). In one of the studies the reduction in breast cancer risk associated with high soy intake was seen among all women (23), whereas two studies were limited to premenopausal women (24,25). A previous report from the SBCS found that high adult soy intake was associated with a reduced risk of breast cancer for women with a higher body mass index (BMI) or with an estrogen receptor/progesterone receptor-positive breast cancer (26).

As indicated in the SBCS, high soy intake appeared to act as a weak estrogen or anti-estrogen only among women with a high BMI (26), IGF-I levels appeared to exert a mitogenic effect on premenopausal breast cancer (7), and estrogen-related hormones and IGF-I levels worked synergistically in the etiology of breast cancer among all women (19). Given that estrogen tends to enhance the action of IGF-I on mammary cell lines (17,19), we hypothesized that high soy protein intake and high IGF-I levels would act synergistically in increasing breast cancer risk. We also hypothesized that there would be a synergistic interaction between high soy protein intake and high IGFBP-3 on breast cancer because IGFBP-3 was positively associated with breast cancer in an earlier SBCS report (7). We collected information from an additional 97 case-control pairs to combine with the 300 case-control pairs from the previous SBCS report (7) to test these hypotheses.

Material and Methods

Detailed methods of this population-based case-control study appeared elsewhere (36). Briefly, all women aged 25–64 yr who were permanent residents of urban Shanghai at the time of diagnosis of first primary invasive breast cancer (August 1996 through March 1998) were eligible for the study. Two senior pathologists histologically confirmed all diagnoses. We used rapid case ascertainment supplemented by the Shanghai Cancer Registry to identify breast cancer cases who had no prior history of cancer. A total of 1,459 breast cancer cases (91.1% of eligible cases) completed a standardized in-person interview. Of potentially eligible cases, 109 refused (6.8%), 17 died prior to the interview (1.1%), and 17 were not located (1.1%).

The Shanghai Resident Registry, a listing of all permanent adult residents of urban Shanghai, was used to randomly select controls. Controls were frequency matched to cases on age (5-yr interval) based on the number of incident breast cancer cases by age group reported to the Shanghai Cancer Registry from 1990 through 1993. Women who did not reside at the registered address at the time of the study were ineligible. A total of 1,556 controls (90.4% of eligible controls) completed a standardized in-person interview. The remaining 166 potentially eligible controls (9.6%) refused participation. Two women died prior to the interview and were excluded.

The study was approved by relevant institutional review boards in Shanghai and the United States. Women were interviewed at hospitals (cases) or at home (cases and controls) by trained interviewers. The subject questionnaire collected information on demographic factors, reproductive and medical histories, family history of cancer, use of oral contraceptives and hormone replacement therapy, diet, physical activity, lifestyle factors, and body size. Adult soy consumption in the previous 5 yr was collected using a 76-item food-frequency questionnaire. Detailed methods of the calculation of soy protein equivalence appears elsewhere (18). Briefly, foods on the questionnaire used to calculate soy protein equivalence based on the Chinese Food Composition Table (37) were tofu, soy milk, fresh soybeans, dried soybeans, soybean sprouts, and other soy products. Weights were applied to these foods to account for the edible portion, the mixture of non-soyfoods, and seasonal variation. The soyfood items were then summed to estimate total soy protein.

After completing the interview, over 80% of women provided fasting blood samples (1,193 cases, 1,310 controls). Detailed methods of blood collection and testing appeared elsewhere (7). Briefly, plasma was separated from samples and stored at –70°C within 6 h of collection. Within the SBCS, a case-control substudy of quantitative biomarkers was conducted utilizing the 397 cases whose fasting blood samples were collected prior to therapy. A total of 397 controls were selected from the pool of controls who provided fasting blood samples. Cases and controls were individually matched on age (within 5 yr), date of blood collection (within 30 days), menopausal status, and, for premenopausal women, menstruation day (within the first 10 days of menstruation during follicular phase or within 3 days of the first 10 days during the follicular or luteal phases). Matched case-control pairs were analyzed in the same batch assay. Plasma IGF-I and IGFBP-3 concentrations were determined with enzyme-linked immunosorbent assay kits available from DSL, Inc. (Webster, TX). Previous studies of IGF-I and IGFBP-3 and cancer have used these methods with good reproducibility (4,38). The intra-assay and interassay precisions measured as coefficients of variation were 1.5–3.4% and 1.5–8.5% for IGF-I and 0.5–1.9% and 1.8–3.9% for IGFBP-3.

χ^2 tests for categorical variables and paired t-tests for continuous variables were used to assess differences in known breast cancer risk factors by case-control status. Spearman

correlation coefficients among controls were computed to evaluate whether levels of IGF-I and IGFBP-3 and soy protein intake were correlated. We used conditional logistic regression to estimate the relative risk of breast cancer associated with IGF-I levels, IGFBP-3 levels, and soy protein intake while controlling for confounders (39). Because these variables were skewed we used the decile distributions among controls and assigned the median of each decile a score for the continuous analysis. We used the tertile distribution among controls to categorize IGF-I levels, IGFBP-3 levels, and soy protein intake in the main effects analysis. The referent group for the main effects analysis was women whose IGF-I level, IGFBP-3 level, or soy protein intake was in the lowest tertile. Due to small numbers in some cells, the median distribution among controls was used to categorize IGF-I levels, IGFBP-3 levels, and soy protein intake in the joint effects analysis. In the joint effects analysis, the referent group was women whose IGF-I or IGFBP-3 levels were less than the median and who consumed less than the median of soy protein. Variables were categorized for all women combined and for premenopausal and postmenopausal women separately. Age, education, family history of breast cancer in a first-degree relative, history of fibroadenoma, leisure physical activity in past 10 yr, BMI, waist-to-hip ratio, parity, age at first live birth, age at menarche, oral contraceptive use, hormone replacement therapy use, and total energy intake were assessed as confounders of the associations between IGF-I levels, IGFBP-3 levels, and soy protein intake and breast cancer. Using a 10% change between unadjusted and adjusted odds ratios (ORs) as evidence of confounding, analyses were adjusted for leisure physical activity in the past 10 yr, parity, and age at first live birth.

Analyses are presented for all women and separately by menopausal status because the effect of some hormonal and growth factor exposures on breast cancer risk is thought to differ by menopausal status. In multiple logistic regression models, we assessed linear trend by treating categorical variables as continuous variables. Interaction terms between IGF-I or IGFBP-3 levels and soy protein intake were included in logistic regression models, and likelihood ratio tests were conducted to examine whether there was evidence of effect modification. We performed an ad hoc analysis that did not involve statistical testing to explore the direction of the effect modification. The OR for the group with high levels of IGF-I or IGFBP-3 and high soy protein intake was divided by the OR for the group with low levels of IGF-I or IGFBP-3 and high soy protein intake. This ratio of ORs was compared with the stratum-specific OR for high levels of IGF-I or IGFBP-3 levels and low soy protein intake.

Results

Table 1 compares known breast cancer risk factors of cases and controls. Compared with controls breast cancer cases were more likely to have a history of fibroadenoma, to have a higher BMI, and to have a later age at first birth and were less likely to have engaged in leisure physical activity in

Table 1. Comparison of Cases and Controls for Selected Risk Factors[a]

	Cases ($n = 397$)[b]	Controls ($n = 397$)[b]	P Value
Age	47.8 ± 7.8	47.6 ± 7.9	0.20
Education (%)			
No formal education + elementary school	12.6	14.6	
Middle school	44.3	43.1	
High school	30.5	31.5	
Profession, college, and above	12.6	10.8	0.74
Breast cancer in first-degree relatives (%)	3.0	1.5	0.15
Ever had breast fibroadenoma (%)	9.1	4.8	0.02
Leisure physical activity in past 10 yr (%)	20.9	29.7	<0.01
Body mass index	23.5 ± 3.3	22.9 ± 3.2	0.02
Waist-to-hip ratio	0.80 ± 0.1	0.80 ± 0.1	0.20
Nulliparous (%)	4.0	3.3	0.57
Age at first live birth (yr)[c]	26.9 ± 4.1	26.3 ± 3.9	<0.01
Menarcheal age (yr)	14.7 ± 1.7	14.9 ± 1.7	0.11
Oral contraceptive use (%)	21.9	25.4	0.24
Hormone replacement therapy use (%)	3.5	3.0	0.68
Postmenopausal (%)	36.9	36.4	0.88
Menopausal age (yr)[d]	48.5 ± 4.5	47.8 ± 4.5	0.12
Usual total energy intake (kcal/day)	1,905.7 ± 470.3	1,862.3 ± 481.9	0.16
Soy protein intake (g/day)	11.5 ± 10.8	12.0 ± 9.8	0.53
IGF-I level (ng/ml)[e]	150.6 (144.5–156.9)	138.5 (133.5–143.8)	<0.01
IGFBP-3 level (ng/ml)[e]	3,963.9 (3,813.6–4,119.5)	3,718.2 (3,586.5–3,854.8)	<0.01

a: Subjects with missing values were excluded from the analysis.
b: Unless otherwise specified, mean ± SD is presented.
c: Among women who had live births.
d: Among women with natural menopause.
e: Geometric mean and 95% confidence interval.

the past 10 yr. Mean soy protein intake did not differ significantly between cases and controls, but women with breast cancer had significantly higher levels of IGF-I and IGFBP-3 than did control women. In comparison with the larger study, cases in the substudy had a significantly smaller waist-to-hip ratio (0.80 vs. 0.81) and older age at menarche (14.7 vs. 14.3), whereas controls in the substudy were more likely to be physically active (29.7% vs. 23.7%) and had an older menarcheal age (14.9 vs. 14.7) (data not shown).

Table 2 shows the Spearman correlation coefficients for IGF-I and IGFBP-3 levels and soy protein intake. The correlation between IGF-I and IGFBP-3 levels in this study was not significant among all controls or by menopausal status nor was there a significant correlation between IGF-I levels and soy protein intake among any control subjects. Although the correlation between IGFBP-3 and soy protein intake was not correlated among all controls, there was a significant negative correlation among premenopausal (r = –0.1389; P = 0.03) and significant positive correlation among postmenopausal controls (r = 0.2123; P = 0.01).

Table 3 presents the ORs and 95% confidence intervals (CIs) for breast cancer associated with IGF-I levels, IGFBP-3 levels, and soy protein intake among all women and by menopausal status. There was an indication of significant associations in the continuous analyses of IGF-I or IGFBP-3

Table 2. Correlations Among IGF-I Levels, IGFBP-3 Levels, and Soy Protein Intake Among All Controls and by Menopausal Status

	Spearman Correlation Coefficient (P Value)		
	IGF-I	IGFBP-3	Soy Protein
All controls			
IGF-I	1.00	–0.0829 (0.10)	–0.0043 (0.93)
Soy protein		–0.0036 (0.94)	1.00
Premenopausal controls			
IGF-I	1.00	0.0240 (0.70)	0.0677 (0.28)
Soy protein		–0.1389 (0.03)	1.00
Postmenopausal controls			
IGF-I	1.00	0.0608 (0.47)	–0.0197 (0.82)
Soy protein		0.2123 (0.01)	1.00

among all women. In addition, there were significant trends of increasing risk associated with increasing levels of IGF-I and IGFBP-3 among all women and by menopausal status. The highest tertile of IGF-I was associated with a twofold increase in breast cancer risk (OR = 2.2; 95% CI = 1.4–3.4) that was seen primarily among women who were diagnosed postmenopausally (OR = 2.2; 95% CI = 0.8–5.8). This pattern held for IGFBP-3 (all women OR = 2.6; 95% CI = 1.5–4.5; postmenopausal women OR = 8.1; 95% CI =

Table 3. Odds Ratios of Breast Cancer Associated With Main Effects of IGF-I Levels, IGFBP-3 Levels, and Soy Protein Intake Among All Women and by Menopausal Status

	OR (95% CI)[a]		
	All Women (397 cases, 397 controls)	Premenopausal Women (250 cases, 252 controls)	Postmenopausal Women (147 cases, 145 controls)
IGF-I levels (ng/ml)			
Continuous	1.1 (1.1–1.2)	1.1 (1.0–1.2)	1.2 (1.0–1.3)
Categorical[b]			
Tertile 1	1.0 (referent)	1.0 (referent)	1.0 (referent)
Tertile 2	1.4 (0.9–2.1)	1.1 (0.6–2.0)	1.1 (0.5–2.6)
Tertile 3	2.2 (1.4–3.4)	1.7 (0.9–3.2)	2.2 (0.8–5.8)
P for trend	<0.001	0.003	0.017
IGFBP-3 levels (ng/ml)			
Continuous	1.2 (1.1–1.3)	1.2 (1.1–1.4)	1.2 (1.0–1.4)
Categorical[c]			
Tertile 1	1.0 (referent)	1.0 (referent)	1.0 (referent)
Tertile 2	1.3 (0.8–2.1)	1.8 (1.0–3.4)	0.5 (0.2–1.3)
Tertile 3	2.6 (1.5–4.5)	1.6 (0.9–2.9)	8.1 (2.5–26.0)
P for trend	<0.001	0.002	0.015
Soy protein (g/day)			
Continuous	1.0 (0.9–1.0)	1.0 (0.9–1.1)	1.0 (0.9–1.1)
Categorical[d]			
Tertile 1	1.0 (referent)	1.0 (referent)	1.0 (referent)
Tertile 2	1.0 (0.7–1.4)	0.9 (0.6–1.4)	1.2 (0.7–2.4)
Tertile 3	1.0 (0.7–1.5)	1.1 (0.7–1.6)	0.7 (0.4–1.5)
P for trend	0.455	0.8	0.485

a: Adjusted for leisure physical activity in past 10 yr, parity, and age at first live birth.

b: Tertiles 1–3 for IGF-I levels for all women were <117.7, 117.7–168.3, and ≥168.4; for premenopausal women were <135.9, 135.9–182.4, and ≥182.5; for postmenopausal women were <96.25, 96.25–130.1, and ≥130.2.

c: Tertiles 1–3 for IGFBP-3 levels for all women were <3,306, 3,306–4,190, and ≥4,191; for premenopausal women were <3,086, 3,086–4,003, and ≥4,004; for postmenopausal women were <3,698, 3,698–4,461, and ≥4,462.

d: Tertiles 1–3 for soy protein intake for all women were <6.96, 6.96–12.21, and ≥12.22; for premenopausal women were <6.89, 6.89–11.85, and ≥11.86; for postmenopausal women were <7.28, 7.28–13.18, and ≥13.19.

2.5–26.0). Soy protein intake was not associated with breast cancer risk. Additional adjustment of the IGF-I analysis for IGFBP-3 and the IGFBP-3 analysis for IGF-I weakened most of these associations (data not shown).

Table 4 shows the effect of increasing IGF-I or IGFBP-3 levels on breast cancer risk for women with low and high levels of soy protein intake among all women and by meno-

pausal status. There were borderline significant associations for the continuous analysis of IGF-I and IGFBP-3 levels among all women regardless of level of soy protein intake. In the categorical analysis, high IGF-I level was associated with an increased risk of breast cancer among all women who consumed high levels of soy protein (OR = 1.7; 95% CI = 1.1–2.6). There were twofold elevations in risk associated

Table 4. Odds Ratios of Breast Cancer Associated With Joint Effects of IGF-I or IGFBP-3 Levels and Soy Protein Intake Among All Women and by Menopausal Status

	OR (95% CI)[a]	
	<9.5 g/day Soy Protein (median)	≥9.5 g/day Soy Protein (median)
All women (397 cases, 397 controls)		
IGF-I levels (ng/ml)		
Continuous	1.1 (1.0–1.2)	1.1 (1.0–1.3)
P for interaction	0.863	0.157
Categorical		
<141.0	1.0 (referent)	0.9 (0.6–1.4)
≥141.0	1.6 (1.0–2.5)	1.7 (1.1–2.6)
P for interaction		0.105
IGFBP-3 levels (ng/ml)		
Continuous	1.2 (1.0–1.4)	1.3 (1.0–4.5)
P for interaction	0.663	0.112
Categorical		
<3741.0	1.0 (referent)	0.9 (0.6–1.4)
<3741.0	2.2 (1.3–3.7)	2.3 (1.4–3.8)
P for interaction		0.265
	<9.1 g/day Soy Protein (median)	≥9.1 g/day Soy Protein (median)
Premenopausal women (250 cases, 252 controls)		
IGF-I levels (ng/ml)		
Continuous	1.1 (0.9–1.3)	1.1 (1.0–1.3)
P for interaction	0.393	0.517
Categorical		
<162.6	1.0 (referent)	1.1 (0.6–2.0)
≥162.6	1.6 (0.9–2.8)	1.7 (1.0–2.9)
P for interaction		0.080
IGFBP-3 levels (ng/ml)		
Continuous	1.4 (1.0–1.9)	1.2 (1.0–1.5)
P for interaction	0.297	0.292
Categorical		
<3,526.0	1.0 (referent)	1.1 (0.6–1.8)
≥3526.0	2.1 (1.0–4.3)	2.5 (1.3–5.0)
P for interaction		0.057
	<10.0 g/day Soy Protein (median)	≥10.0 g/day Soy Protein (median)
Postmenopausal women (147 cases, 145 controls)		
IGF-I levels (ng/ml)		
Continuous	1.1 (0.9–1.5)	1.3 (1.0–1.6)
P for interaction	0.689	0.111
Categorical		
<108.3	1.0 (referent)	0.7 (0.3–1.5)
≥108.3	1.5 (0.7–3.2)	1.5 (0.7–3.3)
P for interaction		0.823
IGFBP-3 levels (ng/ml)		
Continuous	1.2 (0.9–1.6)	1.5 (1.0–2.2)
P for interaction	0.689	0.110
Categorical		
<4,060.5	1.0 (referent)	0.9 (0.4–2.1)
≥4,060.5	2.0 (0.9–4.7)	1.4 (0.7–3.2)
P for interaction		0.176

a: Adjusted for leisure physical activity in past 10 yr, parity, and age at first live birth.

with high IGFBP-3 levels among all women and among premenopausal women regardless of amount of soy protein consumed. Although not significantly different, the OR for high IGFBP-3 levels was higher among premenopausal women with high soy protein intake than among premenopausal women with low soy protein intake, whereas the reverse was true for postmenopausal women. Most of these relations were weakened after additional adjustment for IGFBP-3 or IGF-I levels (data not shown).

The P values for interaction for IGF-I and IGFBP-3 levels and soy protein intake in the categorical analysis were nearly significant among premenopausal women (IGF-I $P = 0.08$; IGFBP-3 $P = 0.57$) but not among postmenopausal women. Among premenopausal women, the direction of the interaction for IGF-I levels was unclear (OR = 1.6; ratio of ORs = 1.6) but appeared to be positive for IGFBP-3 levels (OR = 2.1; ratio of ORs = 2.3). Although there was no evidence of statistical effect modification, the OR and ratio of ORs differed somewhat among postmenopausal women for IGF-I (OR=1.5; ratio of ORs = 2.1) and IGFBP-3 (OR = 2.0; ratio of ORs = 1.6).

Discussion

We found a nearly significant interaction between high soy protein intake and high IGF-I level and breast cancer risk among premenopausal women. The direction of this interaction was unclear, but the negative correlation between IGF-I level and soy protein intake among premenopausal controls would lead one to believe it was negative. This nonsignificant negative interaction was unexpected but could be due to soy inhibiting tumor cell growth stimulated by growth factors. Genistein, the most common isoflavone, has been shown to inhibit the proliferation of breast cancer cells stimulated by epidermal growth factor (40). In contrast, epidermal growth factor and IGF-I have been shown to act synergistically to stimulate breast cancer cell growth (41). Although not significant, the OR and ratio of ORs for IGF-I level and soy protein intake among postmenopausal women were strikingly different and appeared to be positive rather than negative. The mechanism of this potential positive interaction is unknown but could be related to soy's competition as a weak estrogen for receptor sites (20) or to soy acting as an anti-estrogen by reducing estrogen synthesis (21) and increasing sex hormone-binding globulin (22). A previous analysis of the SBCS identified synergistic effects between IGF-I levels and two estrogen-related hormones, estrone and testosterone, on breast cancer risk among women diagnosed premenopausally and postmenopausally (19).

For IGFBP-3 levels, there was a nearly significant positive interaction among premenopausal women. In contrast, the OR and ratio of ORs among postmenopausal women appeared to suggest a negative interaction. The SBCS is one of two studies that identified a stronger association for premenopausal breast cancer with IGFBP-3 levels than with IGF-I levels (6,7). Thus, the nearly significant positive inter-

action among premenopausal women was expected, but the nonsignificant negative interaction among postmenopausal women was not. The lack of significant correlations between these IGF-I and IGFBP-3 levels among premenopausal or postmenopausal controls suggests that the biological mechanisms may have differed by menopausal status.

An alternative explanation for soy enhancing the effect of IGF-I levels on breast cancer is that soy may indirectly affect IGF-I levels because estrogens regulate the expression of IGF-I (42), and selective estrogen receptor modulators such as tamoxifen reduce IGF-I levels (43). To determine whether soy was a confounder or intermediate of breast cancer risk, we assessed the correlation between soy protein intake and IGF-I or IGFBP-3 levels. Soy protein intake was not correlated with IGF-I among any control subjects, but IGFBP-3 was correlated among premenopausal and postmenopausal controls. Nagata et al. (44) did not find a significant correlation between soy and IGF-I or IGFBP-3 levels among premenopausal Japanese women; however, to our knowledge no other studies have assessed these correlations among postmenopausal women. In addition, we found no evidence of confounding after adjusting the IGF-I and IGFBP-3 main effect analyses for soy protein intake. This argues against soy being a confounder or in the causal pathway between IGF-I levels and breast cancer risk but does not rule out this possibility for IGFBP-3.

The nonsignificant positive interaction for IGF-I level and soy protein intake among postmenopausal women, indicating that soy protein may act as a weak estrogen or as an anti-estrogen, is in agreement with laboratory studies showing that estrogen enhanced the effect of IGF-I on breast cancer cell growth (17,18). The nearly significant negative interaction between IGF-I level and soy protein intake among premenopausal women could not be explained by the estrogen–IGF-I hypothesis. In our data, we found that soy protein intake was correlated with estrone sulfate ($r = 0.16$; $P = 0.04$) and sex hormone-binding globulin ($r = -0.14$; $P = 0.07$) levels among premenopausal controls and with testosterone ($r = 0.16$; $P = 0.08$) levels among postmenopausal controls. Soy protein intake was not correlated with any other hormones (dehydroepiandrosterone sulfate, estradiol, estrone, or progesterone), suggesting that the soy protein intake among the study population may not be high enough to alter the estrogen level. More studies are needed to better understand the combined effect of estrogen and growth factor on breast cancer.

This study was not without limitations. Data on IGF-I and IGFBP-3 levels were available for a subgroup of women, reducing statistical power to detect effect modification. IGF-I and IGFBP-3 levels among healthy women in our population were lower than those among Caucasian women in the Nurses' Health Study (4), somewhat limiting the generalizability of our results. A potential explanation for these lower levels is the smaller body size and increased physical activity of Asian women compared with American women. Although blood was collected from cases prior to therapy, there may have been an effect of the disease itself on IGF-I and IGFBP-3 levels. Re-

porting of soy intake is prone to misclassification. A recently completed dietary validation study showed that the correlation of soy protein intake derived from the food-frequency questionnaire that we used in the study and the mean of multiple 24-h dietary recalls was 0.49 (45). Misclassification in assessing soy intake may have compromised our ability to investigate the interactive effects of soy protein intake and IGF-I and IGFBP-3 levels. Change of dietary habits over time, particularly after cancer diagnosis, is another concern. A supplementary questionnaire completed by 295 of 397 controls in the present study indicated that soy consumption reported in the last week was highly correlated with soy consumption reported in the past 5 yr ($r = 0.28$; $P < 0.0001$). Main effects and joint effects analyses comparing women whose diets had not changed with all women were slightly more pronounced but fairly comparable.

Although in vitro (17,18) and in vivo (19) studies of breast cancer have investigated the interaction between estrogen and IGF-I levels, ours is the first in vivo study to investigate the interaction between soy protein, a weak estrogen and anti-estrogen, and IGF-I levels. The relatively high soy consumption among our population compared with the rest of the world made this analysis possible. Additional strengths of this study are its population-based nature and high response rates among subjects (cases: 91%; controls: 90%), which minimizes selection bias. We adjusted for known breast cancer risk factors and evaluated the IGF-I levels, IGFBP-3 levels, and soy protein intake and breast cancer associations in conjunction with menopausal status, a suspected effect modifier of these relations. We also assessed BMI, waist-to-hip ratio, and use of hormone replacement therapy as effect modifiers of the IGF-I–breast cancer association with no evidence of such (data not shown). With the exception of waist-to-hip ratio, age at menarche, and physical activity, we were successful in selecting women for this substudy who were comparable with women from the larger study.

In summary, our results suggest that soy protein intake may modify the effect of IGF-I and IGFPB-3 levels on premenopausal breast cancer risk. Further studies with larger sample sizes are needed to confirm our finding and to understand the biological mechanism of these potential interactions. Should these interactions persist in other studies, intervention studies using soy protein must account for womens' IGF-I and IGFBP-3 levels in their design.

Acknowledgments and Notes

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Appendix 10:Abstract and corresponding poster presentation entitled "Correlation of insulin-like growth factors and c-peptide with energy balance on breast cancer" for poster presentation at NAASO annual meetingVancouver, BC., 2005.

Correlation of insulin-like growth factors and c-peptide with energy balance on breast cancer risk

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Abstract

Despite evidence from many in vitro experiments, limited population-based research has been performed to investigate the insulin interacts with IGFs in the pathogenesis of breast cancer risk. The purpose of this study is to investigate the association of insulin resistance and the joint association with insulin like growth factors (IGF1, IGF2 and IGFBP3) on breast cancer risk. Included in this study, were a subset of subjects (588 case-control pairs from the Shanghai Breast Cancer Study (R01CA64277). Pre-treatment biospecimen and interview data were collected from all breast cancer cases, along with their individually matched controls.

Background

- Previous epidemiologic studies have measured the components of energy intake and energy expenditure independently, rather than in combination. This approach does not provide a cumulative examination of the interrelationship between energy balance and breast cancer risk.

Methods

- The Shanghai Breast Cancer Study, a population-based case-control study was conducted during 1996-1998. The age range of participants was 25-64, with 1459 cases and 1556 controls.
- Subjects for the ancillary study with included 588 cases and 588 controls.
- Cases newly diagnosed with a primary breast cancer.
- Controls matched ±3 years, menopausal status, and date of sample collection ±30 days.

Data Collection and Analysis

- In-person interview measuring traditional breast cancer risk factors and lifestyle variables.
- Anthropometrics: weight, height, waist to hip circumference(two measures taken).
- Biologic sample collection: Fasting blood samples from breast cancer cases collected before cancer therapy.
- Statistical analysis.

Results

Table 1. Comparison of cases and controls on demographics and selected breast cancer risk factors, The Shanghai Breast Cancer Study, 1996-1998.

Table 2. Correlation coefficients among energy balance selected insulin-like growth factors, C-Peptide and endogenous hormones, Shanghai Breast Cancer Study 1996-1998

Table 3. Median levels (25th, 75th percentiles) of IGF-1, IGFBP-3 and C-peptide, SHBG, Estrone by quartiles of energy balance measurements among female controls, Shanghai Breast Cancer Study, 1996-1998.

Reference

Correlation of energy balance, C-peptide, insulin-like growth factors and endogenous sex

hormones on the risk of breast cancer

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Running Title: C-peptide, IGFs, estrogen, energy balance and breast cancer

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Abstract

Our previous studies along with other epidemiological studies suggest energy balance is related to the risk of breast cancer, yet, few studies have investigated potential mediators of this association. We examined if this association could explained by the blood levels of insulin growth factors [IGFs], their binding proteins [IGFBPs], C-peptide and estrogen via endogenous sex hormones in the Shanghai Breast Cancer Study [SBCS], a population-based case-control study of 1459 breast cancer cases and 1556 controls conducted among Chinese women from 1996 and 1998. In-person surveys were used to collect data including a food frequency questionnaire [FFQ], body weight, waist and hip circumference, exercise/sport activity [EA], and occupational activity [OA]. The present analyses consisted of 398 cases and 398 controls whose blood samples were measured for blood levels of IGFs, IGFBP-3, C-peptide, testosterone, estradiol, estrone, estrone sulfate, and DHEAS. We assessed whether body mass index [BMI], waist-to-hip ratio [WHR], physical activity status, and total energy intake may be related to the levels of IGFs, IGFBPs, C-peptide and the endogenous sex hormones and whether the association between energy balance and breast cancer risk may be reduced after adjustment for these biomarkers. BMI and WHR were significantly positively correlated with levels of IGFBP-3 and C-peptide. Adult exercise/sport activity [EA] and occupational activity were significantly negatively correlated with IGF-I. Mean levels of IGF and C-peptide biomarkers by quartile distribution of energy balance measures revealed a significant trend of increasing IGF-I with increasing quartiles of BMI. C-peptide levels increased with increasing quartiles of WHR (p for trend <0.01). What about estrogen results? These results suggest that IGF-I, IGFBP-3 and C-peptide biomarkers partially explain the association between positive energy balance and breast

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cancer risk, but future studies are needed to identify the underlying complex biological

mechanisms of action for breast cancer prevention.

Introduction

Sex steroid hormones, particularly estrogens, play an important role in the pathogenesis

of breast cancer(JNCI 2002) Ecological studies indicate that levels of sex hormones are

substantially higher in Caucasian women than their Chinese counterparts, a population

traditionally at low risk for breast cancer. {Lacey, 2004 74 /id}Parallel differences in traditional

breast cancer risk factors, such as body size measurements and certain lifestyle factors also exist

between Chinese and Caucasian populations. Accumulating results suggest the association

between risk factors such increased adiposity and energy intake and risk for breast cancer may be

mediated by, in part, insulin-like growth factors, c-peptide and endogenous sex hormones.

Insulin may play an etiologic role in association with obesity which increases insulin resistance

and decreases levels of sex hormone binding globulin (SHBG) and therefore elevates estrogen

availability.{Hirose, 2003 78 /id} Therefore, identifying those lifestyle factors that significantly

alter the endocrine and estrogen biomarkers may not only clarify the biological mechanism of

action, but may also be useful in guiding public health prevention measures.

In general, epidemiological studies investigating the correlation between biomarker levels

and energy balance are limited (Newcomb PA, 1996, Kaye, 1991). To date, we have found three

studies conducted in Asian populations that have examined the correlation between level of

biomarker concentration and risk factor for breast cancer (Wu AH, 2002, Nagata, C., 1997).

We recently reported epidemiological evidence that supports an etiologic role of insulin-

like growth factors, c-peptide and endogenous estrogens in the development of breast cancer

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(Yu, Gong, Malin, Boyapati). Furthermore, consistent with the evidence of a positive association, a primary factor related to insulin resistance, overweight status, expressed as a positive energy balance, has been linked to breast cancer risk (Malin, 2005). The purpose of the present sub-study of the Shanghai Breast Cancer case-control study was to investigate the relationship between serum fasting IGF-I, IGF-II, IGFBP-3, C-peptide, endogenous estrogens and positive energy balance with breast cancer risk.

From our previous research on positive energy balance in women in Shanghai we discovered women with lower physical activity levels, higher BMIs, or low physical activity levels and high energy intakes, were at increased risk for breast cancer relative to women with higher activity levels who were lean and/or who reported consuming fewer calories. In this report, we explore possible correlations of C-peptide with IGFs I, II , IGFBP-3 with a positive energy balance.

Methods

The Shanghai Breast Cancer Study was designed to recruit women aged 25-64 who were newly diagnosed with breast cancer between August 1996 and March 1998, and a group of community controls for a population-based case-control study. (Gao *et al*, 2000).All study subjects were permanent residents of urban Shanghai. They had no prior history of cancer and were alive at the time of interview. Through a rapid case-ascertainment system, supplemented by the population-based Shanghai Cancer Registry, 1,602 eligible breast cancer cases were identified during the study period and in-person interviews were completed for 1,459 (91.1%) of the eligible cases. The controls were randomly selected from female residents in urban Shanghai, using the population-based Shanghai Resident Registry and frequency-matched to

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cases by age (5-year intervals). In-person interviews were completed with 1,556 (90.3%) of the 1,724 eligible controls identified.

Trained interviewers measured each eligible subject for her weight, circumference of waist and hips, sitting and standing heights and conducted an in-person interview according to a standard protocol. A structured questionnaire was used to elicit detailed information on demographic factors, menstrual and reproductive history, hormone use, dietary habits, prior disease history, physical activity, tobacco and alcohol use, weight, and family history of cancer. Blood samples were collected from about 83% of the study participants.

Information on usual adult dietary intake was collected using a comprehensive quantitative food frequency questionnaire (FFQ) that covers over 85% of foods consumed in Shanghai. A physical activity questionnaire (PAQ) assessed exercise /sport activity and occupational activity levels. Women could report up to five exercises or sport activities during adolescence (13 to 19 yrs) and adulthood (last 10 yrs). Quantitative exercise/sport data were summarized in terms of intensity (metabolic equivalents (METs), duration (hours/week), years of participation, and average energy expenditure during the period (MET-hours/week/year) using standard methods. (Matthews, 2001). Women also reported their occupational physical activity levels for jobs they held for at least 3 years during their lifetime. For each occupation, participants reported the average time spent in 'standing or walking' and classified the physical exertion of the job according to three activity categories (i.e. heavy, medium, or non-physical work). Summary measures were calculated by multiplying the years spent in each occupation by the specific activity variable, and then summing the results over all jobs.

An individually matched case-control sub-study was built into the Shanghai Breast Cancer Study to increase the comparability of cases and controls in studying quantitative

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biomarkers. For each case whose samples were collected before any cancer treatment, a control was selected from the pool of subjects who completed the study. Controls were individually matched to the index case by age ($\pm$ 3 yrs), menopausal status, and date of sample collection ($\pm$ 30 days). Successful matches were completed for 400 case-control pairs for the present study. To eliminate between-assay variability in case-control comparisons, samples from a matched case-control pair were included in the same batch in the assays of blood IGFs and C-peptide. Three case-control pairs were excluded from all analyses because of lab failure in biomarker assays.

Plasma concentrations of IGF-I, IGF-II, and IGFBP-3 were determined with the use of commercially available ELISA kits (DSL, Inc., Webster, TX). These methods were used in most of previous epidemiological studies(Yu *et al*, 2002). The calibrators used in the assays ranged between 4.5-640 ng/ml for IGF-I, 500-2000 ng/ml for IGF-II, and 2.5-100 ng/ml for IGFBP-3. For IGFBP-3 measurement, plasma samples were diluted at 1:100 in an assay buffer. The intra- and interassay precisions are 1.5-3.4 and 1.5-8.5% of CV respectively, for IGF-I, 4.2-7.2 and 6.3-10.7% of CV for IGF-II and 0.5-1.9 and 1.8-3.9% of CV for IGFBP-3. Each assay has no cross-reaction with other members of the IGF family. Serum C-peptide was measured using an enzymatically amplified one-step sandwich-type ELISA assay kit from Diagnostic System Laboratory, Webster, TX, and the ELISA assay was performed according to the manufacture's instruction. Sample aliquots of 20 μl were pipetted into microtiter wells coated with anti-C-peptide antibodies and incubated with 200μl of a buffered solution of anti-C-peptide antibody conjugated to horseradish peroxidase. Detailed methods of plasma concentrations of testosterone, estradiol, estrone, estrone sulfate and DHEAS measurement appears elsewhere (Boyapati, 2004).

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Both parametric and non-parametric methods were used to analyze the data collected from this study. Log-transformed data were used in the paired student t-tests to compare the mean differences between cases and controls. Pearson correlation coefficients were calculated to evaluate the correlation between dietary factors, physical activity and body size measures and the insulin-like growth factor, binding protein and c-peptide biomarkers. Odds ratios (OR) and 95% confidence intervals (CI) for the upper three quartile groups were derived using conditional logistic regression, compared to the lowest quartile group. Variables were categorized for all women combined. Multivariate analyses were performed to adjust for potential confounding variables.

Results

Comparisons of selected demographic and risk factors between breast cancer patients and their matched controls are shown in Table 1. There were no statistically significant differences between cases and controls with respect to age and education. Although cases had a higher proportion of family history, lower age at menarche, an older age at menopause, and consumed more energy and fat, the differences were not statistically significant. Compared with controls, cases were more physically active, had a higher body mass index (BMI), an older age at the first live birth and consumed more meats. Cases had significantly higher plasma levels of IGF-I, IGF-II, IGFBP-3, and C-peptide than controls. Descriptive statistics on estrogen levels?

Pearson correlation coefficients related body size measurements, dietary/lifestyle measures and physical activity measures to IGF I, II, BP3 and C-peptide are shown in Table 2. Estrogen measures? Body mass index and waist:hip ratio was significantly positively correlated with IGFBP-3 and C-peptide. Adult exercise/sport activity and occupational activity were

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significantly negatively correlated with IGF-I. Adolescent exercise/sport activity and energy

intake were not associated with levels of insulin-like growth factors, binding protein 3 or C-

peptide.

Age and batch-adjusted mean levels of IGF I, II BP3 and C-peptide according to quartile

distributions of select lifestyle measures of energy intake, expenditure and body size are shown

in Table 3. There was a significant trend of increasing IGF-I levels with increasing quartiles of

BMI. IGF-II levels increased with increasing quartiles of BMI. Both IGF-II and C-peptide

increased with increasing quartiles of waist: hip ratio (p trend <0.01). Additional multivariate

analyses were performed to evaluate the joint association of energy balance measures to the

insulin-growth factor, C-peptide and endogenous sex hormone biomarkers. None of these results

changed substantially after adjustment for each of the above biomarkers singly and in

combination (data not shown).

Discussion

To date, we have found three studies conducted in Asian populations that have examined the

correlation between level of biomarker concentration and risk factor for breast cancer (Wu AH,

2002, Nagata, C., 1997, Yu, 2003).

In the study, we found a positive correlation between IGF-II and energy balance measures. It is

known that IGF-II and IGF-I exert their actions at different stages of human growth and are

subject to different regulatory systems{Yu, 2000 36 /id} IGF-II levels are relatively stable during

adulthood whereas IGF-I levels rise and fall substantially with age due to the regulation of

growth hormone.

There are no population-based human studies assessing the interplay of IGFs, C-peptide, sex

steroids and energy balance in relation to breast cancer. Our case-control study is the first human

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study to investigate the correlation between these factors and the findings are in support of

laboratory evidence.

Other underlying mechanism interacting with biomarkers account for some of the relationship or

not at all.

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Table 1. Comparison of cases and controls on demographics and selected breast cancer risk factors,
The Shanghai Breast Cancer Study, 1996-1998.

Characteristics	Cases[a] (n=397)	Controls[b] (n=397)	P-value[d]
Age	47.8±7.8	47.6±7.9	0.20
Education, Elementary or lower (%)	12.56	14.61	0.32
Breast cancer in first degree relatives (%)	1.5	0.75	0.16
Physically active (%)	39.6	35.0	0.005
Body mass index	23.5±3.3	22.9±3.2	0.0186
Waist to hip ratio	0.81+-0.06	0.80+-0.06	<0.001
Age at first live birth (years)[b]	26.9±4.1	26.3±3.9	0.01
Menarcheal age (years)	14.7±1.7	14.9±1.7	0.11
Menopausal age (years)[c]	48.5±4.5	47.8±4.5	0.12
Energy intake (kcal/day)	1905.7±470.3	1862.3±481.9	0.16
Total fat intake (g/day)	37.1±19.3	36.8±15.9	0.79
Total meat intake (g/day)	93.1± 69.2	84.3±53.0	0.04
IGF-I[e]	150.6 (144.52,156.92)	138.5 (133.48, 143.80)	<0.001
IGF-II[e]	820.5 (797.38, 844.19)	798.6 (779.46,820.64)	0.034
IGFBP-3[e]	3963.9 (3813.56,4119.54)	3718.2 (3586.50,3854.76)	<0.0001
C-peptide[e]	1.43 (1.34,1.52)	1.19 (1.12,1.26)	<0.0001

Subjects with missing values were excluded from the analysis
[a]Unless otherwise specified, mean ±SD are presented
[b]Among women who had live births

[c]Among post-menopausal women
[d] P-values were derived from χ^2 tests for categorical variables and paired t-tests for continuous variables
[e]Geometric means and 95% CIs are presented

Table 2. Person correlation coefficients among energy balance and IGF-I, IGF-II, IGFBP-3 and C-Peptide among
controls Shanghai Breast Cancer Study, 1996-1999 Include endogenous hormones in this analysis?

Variable	IGF-I	IGF-II	IGFBP-3	C-Peptide
Adolescent Exercise/Sports (MET-hrs/d/yr)[a]	-0.04/-0.03	0.06/0.06	0.002/0.004	0.02/0.002
Adult Exercise/Sports (MET-hrs/d/yr)[a]	**-0.13**[b]**/-0.14**	0.02/0.02	0.003/0.02	-0.06/-0.08
Occupational Activity (hrs/d/yr.)[c]	**-0.01/-0.01**	0.02/0.02	0.08/0.08	-0.07/-0.11
BMI (kg/m^2)	-0.003/-0.03	0.01/0.009	**0.01/0.09**	**0.22/0.29**
Waist: hip ratio	0.03/0.02	0.07/0.07	0.09/0.09	**0.11/0.17**
Energy Intake (kcal/d)	0.09/0.08	-0.03/-0.03	0.08/0.07	-0.04/-0.04

[a] Unadjusted/log transformed

[b] Bolded values are significant at $p<0.05$

c Occupational activity is measured as the average time spent in standing or walking and classified each into one of
four activity categories (i.e. heavy, medium, or non-physical work).

Table 3. Mean levels of IGF-I, IGF-II, IGFBP-3 and C-peptide by quartiles of energy balance measurements among female controls, Shanghai Breast Cancer Study, 1996-1998.

Variable	IGF-I	IGF-II	IGFBP-3	C-Peptide
Body mass index (kg/m^2) conventional cut points for BMI categories, tertiles?				
≤ 20.70	141.42±46.8	797.24±194.19	3886.52±11476.60	1.25±1.09
20.70-22.79	157.58±54.84	844.12±206.67	3812.83±1311.99	1.34±0.92
22.79-25.1	150.13±55.41	850.40±192.55	4044.22±1746.81	1.58±1.45
>25.1	143.69±61.65	799.70±206.76	4203.27±1631.93	1.80±1.19
p for trend what is the label for this test? [b]	P=0.02	P=0.004	P=0.23	P=0.21
Waist :Hip ratio				
≤ 0.764	148.87±52.80	814.98±197.13	3859.65±1514.85	1.33±1.05
0.764-0.799	147.91±54.23	795.55±200.51	3894.97±1456.24	1.29±0.90
0.799-0.835	147.00±57.36	826.17±210.51	3898.21±1652.43	1.62±1.49
>0.835	150.03±56.38	857.71±194.14	4270.55±1520.75	1.70±1.16
p for trend [b]	P=0.51	P=0.003	P=0.24	P=0.002
Adolescent Exercise/Sports (MET-hrs/d/yr)				
0	148.38±55.33	824.24±195.12	4203.07±1645.96	1.56±1.33
0-2.2	148.31±60.29	788.83±198.68	3741.34±1371.51	1.40±0.97
2.2-7.6	154.43±52.05	846.15±210.56	3715.72±1226.41	1.32±0.88
>7.6	142.06±50.10	835.88±217.38	3677.69±1511.32	1.44±1.03
p for trend [b]	P=0.06	P=0.70	P=0.41	P=0.58
Adult Exercise/Sports (MET-hrs/d/yr)				
0	151.64±55.30	824.98±201.51	3873.77±1534.92	1.51±1.07
0-1.31	146.85±53.65	849.52±206.92	3990.64±1227.41	1.53±2.03
1.31-6.84	140.13±59.40	757.52±168.25	4512.01±1623.31	1.27±0.83
>6.9	133.72±46.54	854.70±218.83	4186.65±166.99	1.40±1.19
p for trend [b]	P=0.32	P=0.03	P=0.15	P=0.42
Occupational Activity (hrs/d/yr.)[c]				
0	152.61±54.90	822.35±202.01	3878.82±1548.10	1.49±1.02
0-2	146.19±60.61	842.76±196.37	3940.44±1219.43	1.85±2.05
2-6	128.58±49.24	776.87±170.15	4652.81±1723.82	1.09±0.56
>6	140.31±50.18	855.79±231.89	4048.19±1471.38	1.30±1.17
p for trend [b]	P=0.21	P=0.04	P=0.38	P=0.08
Energy Intake (kcal/d)				
0	143.00±52.57	855.11±208.60	3801.91±1467.64	1.41±1.01
0-1532.2	144.44±53.35	824.78±194.91	4056.60±1613.80	1.72±1.56
1532.2-2084.9	149.45±48.54	816.09±192.90	3822.71±1299.80	1.31±0.91
>2084.9	155.85±63.60	799.33±206.97	4210.22±1718.70	1.48±1.11
p for trend [b]	P=0.81	P=0.22	P=0.15	P=0.25

[b] test for trend based on quartile ranks
[c] Occupational activity is measured as the average time spent in standing or walking and classified each into one of four activity categories (i.e. heavy, medium, or non-physical work).

Table 4. Joint effects of adult exercise energy intake, and BMI on breast cancer risk
The Shanghai Breast Cancer Study, 1996-1999 n=398 pairs Stratify by menopausal status?
Simply tables for energy balance status, only the high risk group? Remove all of the layers of the
energy balance tables.

Energy Intake (Kcals/day)		BMI (Kg/m²)		Formatted Table
		Low(<21)	Intermediate(21≤BMI<25)	High(≥25)
Adult Exercise/Sports (MET-hrs/d/yr)		**All**	**Women**	
Yes	**Low(<1794)**	**1.0(reference)**	**2.18(0.69-6.91)**	**2.12(0.59-7.6'**
	High(≥1794)	**0.80(0.70-3.27)**	**1.79(0.59-5.45)**	**2.51(0.69-7.6**
No	Low(<1794)	**2.63(0.92-7.54)**	**2.94(1.05-8.23)**	**2.58(0.87-7.6**
	High(≥1794)	1.75(0.60-5.11)	3.19(1.15-8.89)	4.82(1.68-13.8

*Adjusted for age of interview, education, income, history of fibroadenoma, history of breast cancer among first degree relatives, menopausal status, height, and ever had live birth.

Table 4a. Model 1: Joint effects of adult exercise ,energy intake, BMI and
selected endocrine biomarkers on breast cancer risk :
The Shanghai Breast Cancer Study, 1996-1999[*] n=398 pairs

	Energy Intake (Kcals/day)	BMI (Kg/m^2)		
		Low(<21)	Intermediate(21≤BMI<25)	High(≥25)
Model 1: IGF-I only		**All Women**		
Adult Exercise/Sports (MET-hrs/d/yr)				
Yes	**Low(<1794)**	**1.0(reference)**	**2.15(0.67-6.90)**	**2.09(0.57-7.61)**
	High(≥1794)	**0.81(0.19-3.36)**	**1.72(0.56-5.29)**	**2.16(0.64-7.27)**
No	Low(<1794)	**2.85(0.98-8.26)**	**2.84(1.00-8.02)**	**2.62(0.88-7.79)**
	High(≥1794)	1.88(0.64-5.57)	3.27(1.16-9.18)	4.82(1.68-13.86)
Model 1: IGF-II only		**All Women**		
Adult Exercise/Sports (MET-hrs/d/yr)				
Yes	**Low(<1794)**	**1.0(reference)**	**2.12(0.67-6.75)**	**2.13(0.59-7.71)**
	High(≥1794)	**0.75(0.18-3.09)**	**1.65(0.54-5.03)**	**2.52(0.70-7.73)**
No	Low(<1794)	**2.54(0.88-7.30)**	**2.78(0.99-7.81)**	**2.49(0.84-7.34)**
	High(≥1794)	1.77(0.60-5.18)	3.12(1.12-8.70)	4.65(1.61-13.41)
Model 1: IGFBP-3 only		**All Women**		
Adult Exercise/Sports (MET-hrs/d/yr)				
Yes	**Low(<1794)**	**1.0(reference)**	**2.10(0.66-6.67)**	**2.03(0.56-7.38)**
	High(≥1794)	**0.74(0.18-3.06)**	**1.65(0.54-5.03)**	**2.52(0.70-7.73)**
No	Low(<1794)	**2.69(0.93-7.73)**	**2.78(0.99-7.81)**	**2.49(0.84-7.34)**
	High(≥1794)	1.75(0.60-5.13)	3.22(1.15-8.98)	4.64(1.61-13.37)
Model 1: C-peptide only		**All Women**		
Adult Exercise/Sports (MET-hrs/d/yr)				
Yes	**Low(<1794)**	**1.0(reference)**	**2.68(0.79-9.14)**	**2.63(0.69-10.09)**
	High(≥1794)	**0.94(0.20-9.37)**	**2.05(0.64-6.62)**	**2.31(0.65-8.22)**
No	Low(<1794)	**3.05(1.00-9.35)**	**3.31(1.10-9.93)**	**2.76(0.88-8.69)**
	High(≥1794)	1.85(0.59-5.83)	3.72(1.25-11.09)	5.49(1.78-16.91)

[*]Adjusted for age of interview, education, income, history of fibroadenoma, history of breast cancer among first degree relatives, menopausal
status, height, ever had live birth and biomarkers as specified

Table 4b. Model 1: Joint effects of adult exercise ,energy intake, BMI and selected endocrine biomarkers on breast cancer risk :The Shanghai Breast Cancer Study, 1996-1999[*]n=398 pairs

	Energy Intake (Kcals/day)	BMI (Kg/m^2)		
		Low(<21)	Intermediate(21≤BMI<25)	High(≥25)
Model 1: IGF-I, IGF-II, IGBP-3, C-peptide		**All Women**		
Adult Exercise/Sports (MET-hrs/d/yr)				
Yes	**Low(<1794)**	1.0(reference)	2.62(0.76-9.11)	2.53(0.65-9.90)
	High(≥1794)	0.84(0.17-4.05)	1.93(0.59-6.37)	2.24(0.62-8.10)
No	Low(<1794)	3.33(1.07-10.40)	3.26(1.07-9.96)	2.70(0.84-8.86)
	High(≥1794)	2.00(0.62-6.40)	3.85(1.07-4.60)	5.37(1.71-16.84)

[*]Adjusted for age of interview, education, income, history of fibroadenoma, history of breast cancer among first degree relatives, menopausal status, height, ever had live birth and all selected biomarkers

Table 4. Joint effects of adult exercise, energy intake, and BMI on breast cancer risk:
The Shanghai Breast Cancer Study, 1996-1999[*] n=300 pairs

	Energy Intake (Kcals/day)	BMI (Kg/m^2)		
		Low(<21)	Intermediate(21≤BMI<25)	High(≥25)
Adult Exercise/Sports (MET-hrs/d/yr)		**All Women**		
Yes	Low(<1794)	1.0(reference)	2.58(0.67-9.90)	2.48(0.55-11.22)
	High(≥1794)	0.52(0.09-2.89)	1.95(0.52-7.34)	2.81(0.68-11.57)
No	Low(<1794)	3.07(0.86-10.92)	3.32(0.97-11.42)	2.54(0.70-9.21)
	High(≥1794)	2.27(0.63-8.11)	4.45(1.31-15.14)	5.24(1.47-18.60)

[*]Adjusted for age of interview, education, income, history of fibroadenoma, history of breast cancer among first degree relatives, menopausal status, height, ever had live birth and all selected biomarkers

Table 4c. Model 2: Joint effects of adult exercise and sport activity levels, occupational activity levels, energy intake, BMI and selected endogenous estrogens on breast cancer risk :The Shanghai Breast Cancer Study, 1996-1999[*]n=300 pairs

	Energy Intake (Kcals/day)	BMI (Kg/m^2)		
		Low(<21)	Intermediate(21≤BMI<25)	High(≥25)
Model 2: Estradiol only		**All**	**Women**	
Adult Exercise/Sports (MET-hrs/d/yr)				
Yes	**Low(<1794)**	**1.0(reference)**	**2.58(0.67-9.90)**	**2.48(0.55-11.24)**
	High(≥1794)	**0.52(0.09-2.81)**	**1.84(0.49-6.87)**	**2.82(0.69-11.62)**
No	Low(<1794)	**3.05(0.86-10.84)**	**3.29(0.96-11.31)**	**2.38(0.65-8.68)**
	High(≥1794)	2.27(0.63-8.12)	4.43(1.30-15.05)	5.24(1.47-18.59)
Model 2: Estrone only		**All**	**Women**	
Adult Exercise/Sports (MET-hrs/d/yr)				
Yes	**Low(<1794)**	**1.0(reference)**	**2.55(0.66-9.78)**	**2.47(0.55-11.18)**
	High(≥1794)	**0.52(0.09-2.87)**	**1.77(0.47-6.69)**	**3.01(0.72-12.50)**
No	Low(<1794)	**2.91(0.81-10.34)**	**3.17(0.92-10.90)**	**2.22(0.61-8.12)**
	High(≥1794)	2.24 (0.63-7.80)	4.19(1.23-14.24)	5.05(1.42-17.93)
Model 2: Estrone Sulfate only		**All**	**Women**	
Adult Exercise/Sports (MET-hrs/d/yr)				
Yes	**Low(<1794)**	**1.0(reference)**	**2.55(0.66-9.81)**	**2.47(0.55-11.21)**
	High(≥1794)	**0.52(0.09-2.87)**	**1.83(0.48-6.95)**	**2.83(0.69-11.65)**
No	Low(<1794)	**3.04(0.85-10.83)**	**3.28(0.95-11.29)**	**2.42(0.66-8.78)**
	High(≥1794)	2.25(0.63-8.06)	4.41(1.30-15.01)	5.09(1.43-18.14)
Model 2: SHBG only		**All**	**Women**	
Adult Exercise/Sports (MET-hrs/d/yr)				
Yes	**Low(<1794)**	**1.0(reference)**	**2.68(0.79-9.14)**	**2.63(0.69-10.09)**
	High(≥1794)	**0.94(0.20-9.37)**	**2.05(0.64-6.62)**	**2.31(0.65-8.22)**
No	Low(<1794)	**3.05(1.00-9.35)**	**3.31(1.10-9.93)**	**2.76(0.88-8.69)**
	High(≥1794)	1.85(0.59-5.83)	3.72(1.25-11.09)	5.49(1.78-16.91)

[*]Adjusted for age of interview, education, income, history of fibroadenoma, history of breast cancer among first degree relatives, menopausal status, height, and ever had live birth.

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Table 4c. (con't)Model 2: Joint effects of adult exercise, energy intake, BMI and selected
endogenous estrogens on breast cancer risk
The Shanghai Breast Cancer Study, 1996-1999[*] n=300 pairs

	Energy Intake (Kcals/day)	BMI (Kg/m^2)		
		Low(<21)	Intermediate(21≤BMI<25)	High(≥25)
Model 2: Testosterone only		**All**	**Women**	
Adult Exercise/Sports (MET-hrs/d/yr)				
Yes	**Low(<1794)**	**1.0(reference)**	**2.51(0.65-9.68)**	**2.36(0.52-10.73)**
	High(≥1794)	**0.51(0.09-2.82)**	**1.86(0.50-7.05)**	**2.80(0.68-11.54)**
No	Low(<1794)	**3.13(0.88-11.17)**	**3.26(0.55-11.23)**	**2.36(0.65-8.58)**
	High(≥1794)	2.19(0.61-7.87)	4.44(1.30-15.14)	4.88(1.37-17.42)
Model 2: DHEA only		**All**	**Women**	
Adult Exercise/Sports (MET-hrs/d/yr)				
Yes	**Low(<1794)**	**1.0(reference)**	**2.26(0.58-8.77)**	**2.30(0.50-10.54)**
	High(≥1794)	**0.48(0.09-2.70)**	**1.81(0.47-6.91)**	**2.70(0.65-11.23)**
No	Low(<1794)	**3.01(0.84-10.76)**	**3.17(0.92-10.95)**	**2.26(0.62-8.27)**
	High(≥1794)	2.16 (0.60-7.78)	4.17(1.22-14.28)	5.01(1.40-17.89)

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[*]Adjusted for age of interview, education, income, history of fibroadenoma, history of breast cancer among first degree relatives, menopausal
status, height, ever had live birth and selected endogenous estrogens

Table 4d. Model 3: Joint effects of adult exercise, energy intake, BMI and selected endogenous estrogens on breast cancer risk :The Shanghai Breast Cancer Study, 1996-1999[*]n=300 pairs

	Energy Intake (Kcals/day)	BMI (Kg/m²)		
		Low(<21)	Intermediate(21≤BMI<25)	High(≥25)
Model 3: Estradiol, Estrone, Estrone Sulfate, SHBG, Testoterone, DHEA				
		All Women		
Adult Exercise/Sports (MET-hrs/d/yr)				
Yes	Low(<1794)	1.0(reference)	2.22(0.56-8.75)	2.09(0.45-9.73)
	High(≥1794)	0.45(0.08-2.54)	1.70(0.44-6.54)	2.92(0.69-12.33)
No	Low(<1794)	2.79(0.77-10.14)	3.05(0.87-10.65)	2.04(0.55-7.59)
	High(≥1794)	1.98(0.54-7.22)	3.83(1.11-13.26)	4.41(1.21-16.02)

[*]Adjusted for age of interview, education, income, history of fibroadenoma, history of breast cancer among first degree relatives, menopausal status, height, ever had live birth and selected endogenous estrogens.

Table 4. Joint effects of adult exercise and sport activity levels, occupational activity levels, energy intake, and BMI on breast cancer risk[,†]

	Energy Intake	BMI (Kg/m^2)		
		Low(<21)	Intermediate (21<BMI<25)	High(≥25)
Adult Exercise/Sports (MET-			**Women**	
Yes	Low(<1794)	1.0(reference)	0.94(0.52-1.71)	0.92(0.48-1.76)
	High(≥1794)	0.74(0.37-1.46)	0.91(0.51-1.63)	0.81(0.44-1.50)
No	Low(<1794)	1.12(0.66-1.90)	1.25(0.75-2.09)	1.69(0.98-2.89)
	High(≥1794)	0.93(0.54-1.60)	1.47(0.88-2.46)	1.79(1.06-3.05)
P interaction				
Occupational Activity				
Yes	Low(<1794)	1.0(reference)	1.12(0.83-1.52)	1.08(0.72-1.61)
	High(≥1794)	0.85(0.59-1.21)	1.23(0.90-1.68)	1.22(0.85-1.75)
No	Low(<1794)	1.57(0.88-2.79)	1.48(0.88-2.51)	2.82(1.29-6.18)
	High(≥1794)		1.99(1.13-3.53)	
		1.30(0.64-2.65)		1.51(0.64-3.54)
P interaction	0.14			
Adult Exercise/Sports (MET-hrs/d/yr)				
Yes	Low(<1794)	1.0(reference)	0.90(0.35-2.31)	1.15(0.45-2.77)
	High(≥1794)	059(0.19-1.84)	1.03(0.43-2.51)	1.12(0.45-2.77)
No	Low(<1794)	1.36(0.55-3.40)	1.59(0.68-3.73)	2.55(1.07-6.04)
	High(≥1794)	1.01(0.39-2.58)	1.83(0.79-4.25)	2.84(1.21-6.67)
P interaction	0.21			

TITLE: Obesity, Insulin-Resistance, IGFs, and Breast Cancer Risk in African-Americans

CO-LEADERS and KEY PERSONNEL:

Meharry Co-leader: Cui, Yong, MD, MSPH, Assistant Professor
(Proposed starting date: May 1, 2005)

Vanderbilt Co-leader: Zheng, Wei, MD, PhD, Professor of Medicine

ABSTRACT

Breast cancer is the most common malignancy among women and is the second leading cause of cancer-related deaths in the United States. It has been documented that African-American women are more frequently diagnosed with aggressive breast cancer and have a higher mortality rate than their Caucasian counterparts. However, only a limited number of epidemiological studies have been conducted to explore reasons for these racial disparities. In this application, we propose to conduct a case-control study to test several important etiological hypotheses of breast cancer related to obesity and insulin resistance. These hypotheses are new and particularly relevant to the etiology of breast cancer in African-Americans, as a substantially higher percentage of African-Americans are over weight than Caucasian Americans. Approximately 450 cases and 450 controls will be included in the investigation of these hypotheses, of which 600 cases and controls will be recruited in this proposed study and the remaining 300 cases and controls will be recruited as part of the ongoing NCI-funded Nashville Breast Health Study (R01CA100374). We propose to conduct telephone interviews to obtain dietary and other lifestyle information and collect exfoliated buccal cell samples to extract DNA for genotyping assays of polymorphisms in several major genes related to insulin resistance (*INS, INSR, IRS-1, IRS-2, RETN, APM1, LEP, LEPR*) and IGF-1 effects (*IGF-1, IGF1R,* and *IGFBP-3*). Cases newly diagnosed with primary breast cancer within a defined study period (2.5 years) and aged between 25 and 75 years will be identified through the population-based Tennessee Cancer Registry. Controls will be identified using a combined method of DMV (the Tennessee Department of Motor Vehicles) file and RDD (random digit dialing) protocol and frequency matched to cases on age, race, and residency county. This study, one of the first large population-based case-control studies designed specifically to evaluate etiological factors for breast cancer in African-American women, will provide valuable information towards the understanding of the etiology of breast cancer in this underserved population.

SPECIFIC AIMS

Breast cancer is the most common malignancy among women and is the second leading cause of cancer-related deaths in the United States. It has been documented that African-American women are more frequently diagnosed with aggressive breast cancer and have a higher mortality rate than their Caucasian counterparts. However, only a limited number of epidemiological studies have been conducted over the past few decades to explore reasons for the racial disparities in breast cancer incidence and mortality, and the etiology of breast cancer in African-American women remains largely unknown. Currently, we are funded by NCI to conduct the Nashville Breast Health Study (NBHS) (R01CA100374) to recruit breast cancer patients from the Nashville metropolitan area. Given the size of the African-American population in this metropolitan area, we estimate that approximately 150 cases and 150 controls will be recruited into the NBHS, which will have limited statistical power to evaluate etiological hypotheses. In this application, we propose to conduct a case-control study with 450 cases and 450 controls to test several important etiological hypotheses related to the role of obesity and insulin resistance in breast cancer development in African-American women, with a long-term goal of establishing a large-scale epidemiological study of breast cancer to comprehensively investigate both

genetic and environmental factors for breast cancer etiology in the African-American population. The specific aims of this proposed study are:

1) To recruit 300 incident breast cancer cases and 300 age frequency-matched controls from other metropolitan areas of Tennessee that are not covered by the NBHS. Combined with the subjects recruited from NBHS, we will have a total of 450 cases and 450 controls to evaluate etiological hypotheses of breast cancer in African Americans.

2) To conduct telephone interviews to obtain dietary and other lifestyle information and collect exfoliated buccal cell samples and extract DNA from these samples.

3) To analyze genetic polymorphisms in several major genes related to insulin resistance (*INS, INSR, IRS-1, IRS-2, RETN, APM1, LEP, LEPR*) and IGF-1 action (*IGF-1, IGF1R,* and *IGFBP-3*).

4) To perform statistical analyses to test the study hypotheses.

The information obtained from this study will be valuable to refine study instruments and hypotheses for a larger study, which would allow us to comprehensively investigate both genetic and environmental factors of breast cancer etiology among African-American women.

RELEVANCE of PROJECT to MMC/VICC PARTNERSHIP GOALS

This proposed study represents a natural extension of the research collaboration of investigators at both Meharry Medical College (MMC) and the Vanderbilt-Ingram Cancer Center (VICC) in breast cancer epidemiology. Over the past few years, several key investigators of the proposed study have collaborated on multiple epidemiology projects, including the Nashville Breast Health Study (NBHS, R01CA100374), a population-based, case-control study in the Nashville metropolitan area. We propose now to build upon the success in the NBHS to enhance the recruitment of African-American women and the evaluation of breast cancer risk factors in this underserved population. The proposed study will also provide excellent training opportunities for junior investigators (Drs. Cui, Malin, and Adegoke) to further develop their research skills and become independent investigators. Furthermore, the study, if conducted successfully, will provide tremendous resources for many future studies of breast cancer epidemiology and etiology. Therefore, the proposed study is highly relevant to the goals proposed for MMC/VICC Partnership (http://www.mmc-vicc.org).

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY					FROM 03/01/06	THROUGH 02/28/07	
(Meharry Medical College Year 1							

| PERSONNEL (Applicant organization only) | | Type Appt. (months) | % Effort on project | Inst. Base salary | DOLLAR AMOUNT REQUESTED (omit | | |
NAME	ROLE ON PROJECT				SALARY REQUESTED	FRINGE BENEFITS	TOTALS
Yong Cui, MD, MSPH	Principal Investigator	12	20%	75,000	15,000	3,600	18,600
Alecia Malin, DrPH, CHES	Co-Investigator	12	10%	70,000	7,000	1,680	8,680
TBN	senior interviewer	12	50%	38,000	19,000	4,560	23,560
TBN	Interviewer	12	100%	25,000	25,000	6,000	31,000
SUBTOTALS ⟶					66,000	15,840	81,840

CONSULTANT COSTS		
EQUIPMENT (Itemize)		
Freezer		6,200
SUPPLIES (Itemize by category)		
Computers (1)	2,000	
		2,000
TRAVEL	1,500	
		1,500
PATIENT CARE COSTS	INPATIENT	0	0
	OUTPATIENT	0	0
ALTERATIONS AND RENOVATIONS (Itemize by category)		0	0
OTHER EXPENSES (Itemize by category)		
Communications	600	
Questionnare & Forms	1,000	
Participation incentive	5,000	6,600
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD	$	98,140
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	0
	FACILITIES AND ADMINISTRATIVE COSTS	0
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD (Item 7a, Face Page) ⟶	$	98,140
SBIR/STTR Only: FEE REQUESTED		